THE UNIVERSITY *of* EDINBURGH

GENETIC AND ENVIRONMENTAL FACTORS INFLUENCING SUSCEPTIBILITY TO INFECTIOUS AGENTS

C.C. Blackwell

D.Sc.
University of Edinburgh
1993

CONTENTS

Title page	i
Acknowledgements	ii
Introduction	1 – 15
List of Publications	16 – 20
Papers	

ACKNOWLEDGEMENTS

I would like to thank Professor D.M. Weir for his support and encouragement throughout these studies and Dr. A.E. Mourant, FRS for his stimulating correspondence over the past 10 years. I am also most grateful to Dr. John Stewart for his advice and critical discussions and especially to Mrs. V.S. James, Miss. D.A.C. Mackenzie, Mrs. M.K. Cole and Mr. M. Kerr for their individual contributions to these studies and their limitless patience. Much of the work was undertaken with the following postgraduate workers and postdoctoral fellow: Denis Kinane, Peter Winstanley, Susan Thom, Susan May, Sarah Andrew, Ayelet Rahat, Zahra Aly, Abdulrahman Saadi, Aziz Zorgani, Mohammed Raza, Gillian Smith, Steven Essery, Ruth Shinebaum.

The epidemiological investigations could not have been undertaken without the help of clinical colleagues who provided access to patients and laboratory specimens: Dr. Mary Hanson, Central Microbiology Laboratories, Edinburgh; Dr. R.P. Brettle and Dr. J. Gray, City Hospital, Edinburgh; Dr. A.D. Toft and Dr. B.F. Clarke, Department of Medicine, University of Edinburgh; Dr. W.T.A. Todd and Dr. A.K.R. Chaudhuri, Monklands District General Hospital, Airdrie; Dr. R.J. Fallon, Meningococcus Reference Laboratory, Ruchill Hospital, Glasgow; Dr. Kristin Jonsdottir and Dr. Peter Holbrook, University of Iceland; Professor Idris Mohammed, University of Maiduguri, Nigeria; Professor Jenny Kourea-Kremastinou, Athens School of Public Health; Georgina Tzanakaki, Hellenic Institute Pasteur, Athens, Greece; Professor G. Nuki, Rheumatology Unit, Western General Hospital; Dr. A.A.M Gibson, Department of Paediatric Pathology, Royal Hospital for Sick Children, Glasgow.

The financial support of the following groups is gratefully acknowledged: The Scottish Home and Health Department; The Scottish Hospitals Endowment Research Trust; The Medical Research Council; The Wellcome Trust; Glaxo Research Ltd; The Meningitis Trust; The Arthritis and Rheumatism Council; TENOVUS Scotland; The Scottish Cot Death Trust; The Meningitis Association of Scotland; Sir Samuel Scott of Yews Trust; Sir Stanley and Lady Davidson Research Fund.

GENETIC AND ENVIRONMENTAL FACTORS INFLUENCING SUSCEPTIBILITY TO INFECTIOUS AGENTS

"Much more attention should be given to the combined effects of blood group and secretor status on susceptibility to bacterial infection."

A.E. Mourant, FRS (Blood Relations)

INTRODUCTION

Almost as soon as the ABO blood groups had been identified in 1900, publications appeared on the distribution of these antigenic phenotypes among patients with a variety of diseases and conditions. In the introductory chapter of Blood Groups and Diseases, Mourant emphasized that it was only in 1953 that the first convincing evidence was found for an association between group A and carcinoma of the stomach. Additional studies by C.A. Clarke and R.B. McConnell found associations between other forms of cancer and ulcers with ABO blood groups and with the ABH secretor status.

Much of the earlier work was severely criticised by Wiener on the basis that if a hypothesis could not be put forward to explain the observed associations, they were probably due to chance. The associations reported for ABO blood groups and infectious diseases were excluded from these criticisms because many microorganisms express antigens cross-reactive with A,B and H (the antigen of blood group O). It was suggested that anti-A and anti-B isohaemagglutinins might act as "natural" antibodies in a non-immune host against organisms expressing these antigens.

Although the ABO blood groups and secretor status are stable, easily and inexpensively determined genetic markers, further investigations of the initial epidemiological findings were not pursued for a variety of reasons. Different groups found different ABO blood group associations for the same infectious disease. These might be explained by antigenic variation

in the strains causing the outbreaks studied or differences in populations, environmental factors, size or classification of patient categories. Statistical methods used might also be important considerations as some environmental factors can mask the contribution of blood group or secretor status which can only be identified by use of multivariate analyses [45]. The interest in the genes of the major histocompatibility group (MHC) generated by the advent of tissue and organ transplantation very much overshadowed studies of blood group antigens with reference to immunity and infection. I think the main reason these observations were not pursued is that only one hypothesis, that anti-A and anti-B could act as "natural" antimicrobial antibodies, was put forward to explain the epidemiological observations.

Research begun in the 1970's identified the important role of protein-carbohydrate interactions in phagocytosis of microorganisms by non-immune hosts and the lectin-mediated binding of potentially pathogenic microorganisms to carbohydrate receptors on host epithelial cells. These studies, in conjunction with the hypothesis that infectious diseases might contribute to the differences in distributions of blood group antigens among different populations, were the starting points for my work on the role of blood groups and secretor status in susceptibility to infectious agents. In addition to ABO blood groups and secretor status, epidemiological data for large patient and control populations collected in my studies have provided a means to examine important environmental factors proposed to contribute to susceptibility to infectious agents, e.g., smoking and passive exposure to cigarette smoke.

As new data suggested that microorganisms might be involved in conditions other than classical infectious diseases such as peptic ulcers and insulin dependent diabetes, I have been invited by clinical colleagues from a number of disciplines to join in their investigations. Most recently I have applied the techniques developed for studies on bacterial meningitis to examination of sudden infant death syndrome (SIDS) as it has been suggested that microorganisms might play a role in some cot deaths.

ABO BLOOD GROUPS AND SUSCEPTIBILITY TO INFECTION

The model chosen to examine the role of ABO blood groups and susceptibility to infection was the increased proportion of group B individuals among those with gonorrhoea [1,4,6-14,16]. Three hypotheses were tested:

1. The anti-B isohaemagglutinins found among O and A individuals are bactericidal antibodies for some strains of Neisseria gonorrhoeae.

2. The anti-B isohaemagglutinins enhance opsonization of gonococci by neutrophils or mononuclear phagocytes.

3. The epithelial cells from group B individuals bind greater numbers of gonococci compared with cells from groups A or O.

Bactericidal activity of normal human serum and isohaemagglutinins

Although gonococci adsorbed isohaemagglutinins, natural bactericidal activity of normal human serum was not associated with either anti-A or anti-B. The important factor was the lipopolysaccharide (LPS) structure of the bacteria. Strains could be divided into those with "simple" or "complex" LPS structure based on sensitivity to R type pyocins from Pseudomonas aeruginosa [1]. Those with the complex LPS obtained from localized genital infections were killed equally efficiently by sera from donors of blood groups A or B while those with the "simple" LPS were uniformly resistant [8]. This observation was exploited with colleagues from Birmingham in studies of induced serum resistance of gonococci [14]. These studies were the first indication that alteration in LPS was involved in this phenomenon. Some isolates from disseminated gonococcal infections expressed the complex LPS, but they were resistant to normal sera, indicating that there were at least 2 mechanisms of serum resistance

in these bacteria.

Mice inoculated with strains expressing either simple or complex LPS produced bactericidal antibodies to the strains of the same LPS type used for inoculation and also to those with the opposite LPS type. This did not occur in humans. Patients with gonorrhoea infections had substantial bactericidal titres to strains with the complex LPS; however, there was no significant activity against 2 strains with the simple LPS tested [12]. This observation emphasized that the immune responses of humans and laboratory animals to microorganisms that are strictly human pathogens might differ significantly; consequently, all studies on the immune responses were examined with human sera or saliva samples.

Opsonizing activity of isohaemagglutinins

Gonococci can be bound to neutrophils and monocytes in the absence of serum by lectin-like interactions that can be inhibited with simple sugars [13]. Following opsonization of the bacteria with sera from donors of different blood groups, there was no difference in attachment of the gonococci to neutrophils [7].

There was no difference in binding of unopsonized bacteria to monocytes from the 4 ABO blood groups. There was, however, a significant increase in binding of gonococci opsonized with sera from donors of blood groups A, B or O but not with sera from AB donors. Group B monocytes bound the greatest number of bacteria; and adsorption of isohaemagglutinins reduced binding to that observed for unopsonized bacteria [9].

Novotny and his colleagues suggested gonorrhoea is a "disease of the human macrophage." In their studies, gonococci were found in pus surrounded by debris and granules derived from monocytes. It was suggested that these materials protect the bacteria from serum and phagocytic activity, and these "infectious units" are important in the survival of the

bacteria in the host. In the context of this hypothesis, our results suggested that the increased association of gonococci with B monocytes might result in the increased probability of formation of infectious units in group B individuals and that this might contribute to the excess of group B among patients with gonorrhoea [11].

Binding of gonococci to epithelial cells of blood group A or B

Although the binding of gonococci to uroepithelial cells varied with different quarters of the menstrual cycle, cells from group B women bound more gonococci than those of A. This suggests that the B antigen might be one of the receptors for some of these bacteria [16]. These studies on gonorrhoea have not been pursued further as at this stage outbreaks of meningococcal disease had appeared in Western Europe and I was urgently persuaded by colleagues working in infectious diseases to apply these methods and techniques developed to the problem of bacterial meningitis.

SECRETOR STATUS AND SUSCEPTIBILITY TO INFECTIOUS AGENTS

There are two phenotypes associated with the secretor (Se) gene. Secretors have in their body fluids such as saliva or nasal secretions the carbohydrate determinants of their ABO blood group antigens, much of which is bound to protein molecules. Non-secretors do not express these antigens in their body fluids. The secretor gene is found on chromosome 19, and it is inherited in a Mendelian dominant pattern. Approximately 75-80% of most populations are secretors and 20-25% express the recessive non-secretor phenotype. There can be significant variations from these proportions in different ethnic groups such as Lapps, Eskimos and American Indians in which over 95% are secretors. The genetics and biochemistry of the ABO blood groups and the interactions between the secretor and Lewis blood group genes have been studied in great detail. The expression of these antigens on cells and in body fluids of secretors and non-secretors is summarized in the table on next page.

Blood group antigens on cells and in body fluids

of secretors and non-secretors

	Secretors		Non-secretors	
BG antigen	cells	secretions	cells	secretions
H (A/B)	+	+	+	–
Lewis[a]	+/–	+/–	+	+
Lewis[b]	+	+	–	–

It was the differences in the distributions of these antigens that suggested some of the hypotheses proposed to explain susceptibility of non-secretors to a variety of bacterial and yeast infections.

Epidemiological Studies

When Mourant and his colleagues published Blood Groups and Diseases in 1978, almost 25 pages were devoted to the studies on ABO blood groups and infectious diseases. Less than 1 page covered the studies on secretor status and infection, mainly the excellent studies from the 1950's and 1960's on rheumatic fever by Glynn and Holborow. With the exception of gonorrhoea [6], among patients with the bacterial diseases investigated, my studies have found a significant excess of non-secretors: recurrent urinary tract infections [5]; kidney scarring associated with urinary tract infection [32]; meningococcal disease [18]; invasive disease due to pneumococci [18]; invasive disease due to type b Haemophilus influenzae [19]. A similar pattern was found among patients with oral or vaginal infections due to yeasts [34]. Patients with diabetes often have problems due to yeast infection and were studied as a model for these infections in the immunocompromised host. The large cohort of diabetic patients examined and multivariate analyses allowed dissection of the very different genetic and

environmental factors contributing to carriage of yeasts [54] and to oral disease in individuals with insulin dependent compared with those with non-insulin dependent diabetes [45].

Respiratory virus infections have been suggested to be one of the factors associated with bacterial meningitis. When my group tested the hypothesis that non-secretors would also be over-represented among patients with respiratory virus diseases, the first associations between secretion of blood group antigens and disease susceptibility were identified: respiratory disease caused by influenza A and B, rhinovirus, respiratory syncytial virus (RSV), ECHO viruses [46]; and heterosexual transmission of the human immunodeficiency virus [47].

The suggestion that autoimmune diseases might be triggered by infectious agents led to surveys of secretor status among patients with conditions for which infectious triggers had been postulated. A significantly higher proportion of non-secretors were found among patients with some rheumatic conditions [21], insulin dependent diabetes mellitus (IDDM) but not among those with non-insulin dependent diabetes (NIDDM) [23], and Graves' disease [26]. Studies by other groups that identified Helicobacter pylori in patients with peptic ulcer resulted in reassessment of blood groups and secretor status with reference to presence of these bacteria [44].

Susceptibility of non-secretors to infectious agents

Since there were no apparent associations between ABO blood groups and the bacterial or fungal diseases for which there was an association with non-secretion, the following hypotheses proposed were limited to factors common to all blood groups.

1. If H antigen found on the epithelial cells of all individuals except the very rare Bombay phenotype can act as a receptor for some microorganisms, the H antigen and Lewis^b

antigens found in body fluids of secretors might bind to the bacterial adhesin, reducing the probability of colonization of secretors.

2. The Lewis^a antigen found in consistantly greater amounts on the epithelial cells of non-secretors might act as a receptor for some microoganisms, enhancing the probability of colonization of non-secretors.

3. As the gene for the third component of complement is found in the same linkage group as that of the secretor gene, there might be differences in levels of C3 in the sera of secretors compared with non-secretors.

4. The lower levels of secretory IgA reported for non-secretors might reflect impaired mucosal responses and contribute to a compromise of epithelial defences.

The role of H as a receptor.

Although boiled saliva from secretors did reduce binding of candida to epithelial cells [34], there is little evidence to support the hypothesis that H antigen is a receptor. Binding of candida blastospores to cells of non-secretors was greater; therefore, the binding of the blastospores to epithelial cells could not be proportional to the amount of H expressed. Cells of non-secretors express significantly lower levels of H than cells of secretors due to the presence of H type 1 adsorbed from body fluids [42]. A similar pattern of increased binding to non-secretor cells has been observed for staphylococci [56].

The role of Lewis^a antigen as a receptor.

Although Lewis^a antigen is found on cells of >95% of individuals, it is usually present in greater quantities on those of non-secretors. Binding of candida blastospores [35] and some toxigenic strains of Staphylococcus aureus to cells of non-secretors was greater than to that

of secretors [51]. Polyclonal antibodies to Lewis^a antigen inhibited binding of the yeast to cells of non-secretors but not to those of secretors; but, pretreatment of cells of secretors or non-secretors with polyclonal anti-Lewis^b had no effect on binding of the blastospores [35]. Binding of staphylococci was inhibited by pre-treatment of host cells with monoclonal anti-Lewis^a antibody; and binding of staphylococci to epithelial of secretors and non-secretors was significantly correlated with the amount of Lewis^a detected on the epithelial cells [57].

The amount of Lewis^a expressed on the cells of an individual appears to be constant. Although epithelial cells of non-secretors usually bind high quantities of anti-Lewis^a antibody and those from individuals who lack the Lewis gene bind very low amounts, the amount of Lewis^a detected on epithelial cells from secretors is highly variable [51]. This might explain why studies comparing binding of microorganisms to secretor and non-secretor cells have given non-significant results in many cases. Even if the microorganism had an adhesin that recognized Lewis^a, differences in binding would not be detected if donors were matched only for sex and age but not the amount of Lewis^a expressed. If there are adhesins that bind to Lewis^a present on a variety of microorganisms, one of the factors contributing to susceptibility of infants to colonization by potentially pathogenic bacteria could be the fact that both secretors and non-secretors express easily detectable quantities of Lewis^a during the first few months of life [46,51].

Complement levels of secretors and non-secretors

Among healthy participants in a large survey for meningococcal carriage [24], the levels of C3 were lower among non-secretors, but not significantly so; however, among the 8 donors whose C3 levels were below the lower limits of the normal range, 7 were non-secretors [30]. It had been reported that among patients with insulin dependent diabetes mellitus (IDDM), but not those with non-insulin dependent diabetes (NIDDM), the levels of C3 and C4 were lower than that of non-diabetic individuals. As my studies had found a higher proportion of

non-secretors among patients with IDDM [23], C3 and C4 levels of these patients were examined. Both these components were significantly lower among non-secretors with IDDM [22]. The implications of these findings are unresolved, as I have not had resources to investigate the biological activities of C3 from these individuals or the prevelence of the F and S variants.

The humoral immune responses of secretors and non-secretors

Based on early reports that non-secretors had lower levels of secretory IgA and serum IgA and IgG, we examined the hypothesis that these lower levels of IgA might reflect an impaired immune response to some microorganisms. None of our studies have found evidence to confirm the earlier reports. Among women treated for 20 years or more for recurrent urinary tract infections, the levels of total serum IgA and IgG of non-secretors were significantly higher than those of secretors [20,38]. Among saliva specimens from children examined in the Stonehouse survey, we found no association between secretor status and total salivary IgA; there were, however, significantly higher levels of IgA among those children who were carriers of meningococci, but not those from whom Neisseria lactamica was isolated [36].

Material collected in association with the outbreak of meningococcal disease at the Airdrie Academy [39] provided the opportunity to measure both total salivary and serum levels of IgA, IgG and IgM and the amounts of antibodies specific for the neisseriae. Again, we found no evidence for differences in levels of total or specific serum immunoglobulin between secretors and non-secretors. There were no differences in the levels of total or specific salivary IgA or IgG; however, there were significant differences in both total and specific levels of salivary IgM. Non-secretors had lower levels compared with secretors. Although immunoglobulin levels were raised among carriers, the effect of secretor status was still significant [53]. As children under the age of 12 months have little secretory IgA and are dependent on their secretory IgM for protection of their mucosal surfaces, these differences

in IgM levels might contribute to the apparent increased susceptibility of non-secretor infants to colonization or disease.

ENVIRONMENTAL FACTORS CONTRIBUTING TO SUSCEPTIBILITY TO INFECTION

The epidemiological studies of secretor status and oral candidiasis and secretor status and meningococcal disease have also identified environmental factors that contribute to increased carriage of these two potential pathogens.

Oral candidiasis and carriage of yeasts

Because non-secretors were found to be over-represented among patients with oral or vaginal yeast infections [34], the role of secretor status in susceptibility to these infections among immunocompromised individuals was investigated. Patients with diabetes were chosen as a model because the prevalence of these infections have been reported to be increased in this group. They could be assessed more easily and safely than those undergoing immunosuppressive therapy or those with HIV infection; and there is a large enough population to obtain statistically relevant numbers, particularly as 18 genetic and environmental variables were examined. The aetiology and pathogenesis of IDDM and NIDDM differ significantly, and these studies were the first designed to assess risk factors leading to oral candidiasis and carriage of yeasts in these two groups of patients. One of the important observations was that oral candidiasis is not always associated with the presence of Candida albicans [45].

The risk factors for both disease and carriage differed significantly for patients with IDDM compared with those with NIDDM. Non-secretion was a significant factor associated with disease and carriage, but only among patients with NIDDM. In addition, the studies identified other factors such as the importance of glycaemic control and emphasized the importance of multivariate analyses in these studies [45,54].

Bacterial meningitis

Smoking and passive exposure to cigarette smoke

Smoking was identified as a risk factor for meningococcal carriage among the children in the survey of the Airdrie Academy [39]. As smoking and passive exposure to cigarette smoke have both been implicated as contributing to meningococcal carriage, studies were carried out in Greece where there are fewer social restrictions on smoking than in the United Kingdom. Among the Greek military recruits smoking was a highly significant factor associated with carriage. Smoking was found to be associated with socioeconomic category; the pattern was similar to that observed in the United Kingdom. Among men, those in professional classes were less likely to smoke than those with fewest years of formal education [49].

In a study of carriage of meningococci among Greek school children, the mother's smoking was significantly associated with carriage of these bacteria. In contrast to the pattern observed for women in the United Kingdom where smoking is associated with socioeconomic group as it is for men, approximately a third of Greek women in all socioeconomic groups smoked. This might explain the observation that carriage rates for meningococci were similar in the two areas of Athens, one in which socioeconomic indicators were high and one in which they were significantly lower [58,59].

Gonococcal infection and meningococcal disease

As there appeared to be significant cross-reactivity between meningococci and gonococci, it was suggested that gonococcal infection might increase bactericidal activity against meningococci. The results refuted this hypothesis. Compared with sera from women attending antenatal clinics (a population in which the prevalence of gonorrhoea is very low), there was little bactericidal activity detected against serogroupable meningococci in the sera

of patients of both sexes with gonorrhoea. This might be a factor contributing to the secondary peak of invasive meningococcal disease among teenagers and young adults, particularly military recruits [10].

Viral infections as predisposing factors for bacterial diseases

Another factor suggested to contribute to susceptibility to bacterial meningitis is respiratory viral infection. The studies of Greek military recruits suggested that virus infections in general did not increase carriage of meningococci. Although the proportion of recruits with respiratory infections was greatly increased during the winter screening (approximately 34-46%), the proportion of carriers of meningococci (24-31%) was not significantly increased compared with that observed during the summer (18-30%) when the proportion of recruits with symptoms of respiratory infection was much lower (approximately 5-7%) [59]. If the general effects of viral infection did not enhance carriage, another hypothesis is that some viruses induce specific antigens in the epithelial cells of the host that act as additional receptors for meningococci, enhances their binding to the virus infected cells.

Several observations led to the use of respiratory syncytial virus (RSV) as a model. It affects most children by the age of 2 years; and, it occurs most frequently during the winter months when meningitis is also most common. To test the hypothesis that RSV might be involved in these diseases, binding of several strains of meningococci and of type b H. influenzae to uninfected HEp-2 cells and these cells infected with RSV was compared. There was a significant increase in binding of these bacteria to the virus infected cells [56]; and, this pattern was also observed for both toxigenic and non-toxigenic strains of S.aureus [56, 57].

Sudden Infant Death Syndrome (SIDS)

The methods developed for the study of bacterial meningitis have recently been applied to cot deaths. Analysis of epidemiological studies by other groups with reference to our laboratory studies suggest the following hypothesis. SIDS occurs during the vulnerable period when maternal antibodies are declining and the infants's immune system is immature. It has been suggested that colonization by toxigenic bacteria during this period might be responsible for some of these infant deaths. Staphylococci and group A Streptococcus pyogenes produce pyrogenic toxins that result in high fever; and they are "superantigens" having a number of other physiological effects including induction of powerful cytokines such as TNF and interleukin 1.

The coincidence of the peak of cot deaths with the reported peak in the proportion of infants expressing Lewis^a antigen prompted studies on the role of this antigen as a receptor for toxigenic staphylococci. The enhanced binding of staphylococci to RSV-infected cells suggested a second factor that might contribute to colonization or density of colonization of infants by these bacteria [51,56].

These findings assessed in the context of epidemiological studies on cot deaths suggested a scheme by which various factors might contribute to SIDS. The pyrogenic toxins are produced by these bacteria between 37-40°C; and the amount of toxin is increased at higher temperatures. If the infant is colonized by toxigenic bacteria and its body temperature is increased by viral infection, over-wrapping with clothing or bedding or by being placed in the prone sleeping position, this might result in significantly increased toxin production. The toxin could contribute to some of the mechanisms proposed to be involved in these deaths: heat shock; or, through interleukin 1 induction, the deep sleep state associated in some instances with apnoea [51,56,57].

The studies summarized in this introduction have identified both genetic and environmental factors that contribute to susceptibility to infectious agents. The hypotheses proposed to explain the epidemiological studies have provided several new insights into the host-parasite interactions involved in some infectious diseases and provided new approaches to examination of cot deaths.

Perhaps the major contribution of the work on blood group and secretor status will be identification and characterization of glycoprotein adhesins that use these carbohydrate cell surface components as receptors. One of the major problems in development of vaccines against encapsulated bacteria that cause disease in young children is that infants and children under the age of 5 do not make long-lasting or effective immune responses to polysaccharide antigens. Serotype protein antigens of meningococci are under investigation as vaccines, but will be of limited value. Work in progress in my laboratory has isolated from outer membranes of meningococci and staphylococci protein components with a molecular weight of approximately 60,000 daltons reactive with Lewis^a. If the material can be shown to be immunogenic in humans it might be a valuable candidate for vaccines against serogroup B meningococci.

LIST OF PUBLICATIONS

Dr C.C. Blackwell

1. Blackwell, C.C., Young, H. and Anderson, I.S. 1979. Sensitivity of Neisseria gonorrhoeae to partially purified pyocines of Pseudomonas aeruginosa and a possible approach to epidemiological typing. Journal of Medical Microbiology, 12: 321–335.

2. Blackwell, C.C. and Law, J.A. 1981. Typing of non-serogroupable Neisseria meningitidis by means of sensitivity to R-type pyocines of Pseudomonas aeruginosa. Jouunal of Infection, 3: 370–378.

3. Blackwell, C.C., Winstanley, F.P. and Telfer Brunton, W.A. 1981. Sensitivity of thermophilic bacteria campylobacters to R-type pyocines of Pseudomonas aeruginosa. Journal of Medical Microbiology, 15: 247–251.

4. Kinane, D.F., Blackwell, C.C., Weir, D.M. and Winstanley, F.P. 1982. Phagocyte recognition of Neisseria gonorrhoeae. Journal of Clinical and Laboratory Immunology, 9: 168–172.

5. Kinane, D.F., Blackwell, C.C., Brettle, R.P., Weir, D.M., Winstanley, F.P. and Elton, R.A. 1982. Blood group, secretor state and susceptibility to recurrent urinary tract infection in women. British Medical Journal, 285: 7–9.

6. Kinane, D.F., Blackwell, C.C., Winstanley, F.P. and Weir, D.M. 1983. Blood group, secretor status and susceptibility to infection by Neisseria gonorrhoeae. British Journal of Venereal Diseases, 59: 44–46.

7. Blackwell, C.C., Kowolik, M.J., Winstanley, F.P., Kinane, D.F. Weir, D. M., Law, J.A. and Ho Wang Chok, Y.L. 1983. ABO blood groups and susceptibility to gonococcal infection I. Factors affecting phagocytosis of Neisseria gonorrhoeae. Journal of Clinical and Laboratory Immunology, 10: 173–178.

8. Winstanley, F.P., Blackwell, C.C., Weir, D.M. and Kinane, D.F. 1983. ABO blood groups and susceptibility to gonococcal infection. II. The relationship of lipopolysaccharide type to gonococcal sensitivity to the bactericidal activity of normal human serum. Journal of Clinical and Laboratory Immunology, 11: 27–32.

9. Kinane, D.F., Blackwell, C.C., Winstanley, F.P., Weir, D.M. and Elton, R.A. 1983. ABO blood groups and susceptibility to gonococcal infection. III. role of isohaemagglutinins in increased association of Neisseria gonorrhoeae to monocytes from blood group B individuals. Journal of Clinical and Laboratory Immunology, 12: 83–86.

10. Winstanley, F.P., Blackwell, C.C., Weir, D.M. and Kinane, D.F. 1983. Gonorrhoea: a predisposing factor for meningococcal disease? Lancet ii: 1134–1135.

11. Blackwell, C.C., Kinane, D.F., Winstanley, F.P., Kowolik, M.J. and Weir, D.M. 1984. The role of the monocyte in gonococcal infection. Proceedings of the Tissue Culture and Reticuloendothelial Society. Akademia Kaido, pp 199–203.

12. Blackwell, C.C., Winstanley, F.P. and Weir, D.M. 1984. Absence of bactericidal antibodies against group I lipopolysaccharide determinants of Neisseria gonorrhoeae during human infection. Journal of Medical Microbiology, 17: 353–356.

13. Kinane, D.F., Weir, D.M., Blackwell, C.C. and Winstanley, F.P. 1984. Binding of Neisseria gonorrhoeae by lectin-like receptors on human phagocytic cells. Journal of Clinical and Laboratory Immunology, 13: 107-110.

14. Winstanley, F.P., Blackwell, C.C., Tan, E.L., Patel, P.V., Parsons, N. J., Martin, P.V.M. and Smith, H. 1984. Alteration of pyocin-sensitivity pattern of Neisseria gonorrhoeae is associated with induced resistance to killing by human serum. Journal of General Microbiology, 130: 1303-1306.

15. Blackwell, C.C., Andrew, S., May, S.J., Weir, D.M., MacCallum, C. and Brettle, R.P. 1984. ABO blood group and susceptibility to urinary tract infection: no evidence for involvement of isohaemagglutinins. Journal of Clinical and Laboratory Immunology 15: 191-194.

16. Blackwell, C.C., Winstanley, F.P., Weir, D.M. and Kinane, D.F. 1985. Host-parasite interactions influencing susceptibility to diseases caused by the pathogenic Neisseria species. Fourth International Symposium on the Pathogenic Neisseria Species. American Society for Microbiology, Washington, D.C. 452-455.

17. Winstanley, F.P., Blackwell, C.C. and Weir, D.M. 1985. Factors influencing susceptibility to meningococcal disease. Biomedicine and Pharmacotherapie, 39: 167-170.

18. Blackwell, C.C., Jonsdottir, K., Hanson, M.F., Weir, D.M., Brettle, R.P., Todd, W.T.A., Chaudhuri, A.K.R. and Mathew, B. 1986. Non-secretion of ABO blood group antigens and susceptibility to infection with Neisseria meningitidis and Streptococcus pneumoniae. Lancet, ii: 284-285.

19. Blackwell, C.C., Jonsdottir, K., Weir, D.M. and Hanson, M.F. 1986. Non-secretion of ABO blood group antigens predisposing to infection by Haemophilus influenzae. Lancet, ii: 687.

20. Blackwell, C.C., May, S.J., Brettle, R.P., MacCallum, C.J. and Weir, D.M. 1987. Secretor state and immunoglobulin levels among women with recurrent urinary tract infections. Journal of Clinical and Laboratory Immunology, 22: 133-137.

21. Shinebaum, R., Blackwell, C.C., Forster, P., Hurst, N.P., Weir, D.M. and Nuki, G. 1987. Non-secretion of ABO blood group antigens: a new host susceptibility marker for the spondarthropathies. British Medical Journal 294: 208-210.

22. Blackwell, C.C., Winstanley, F.P., Weir, D.M. and Kinane, D.F. 1987. Lipopolysaccharide structure and serum sensitivity of non-serogroupable Neisseria meningitidis. Journal of Clinical and Laboratory Immunology 24: 29-32.

23. Blackwell, C.C., James, V.S. and Weir, D.M. Gemmill, J.D., Patrick, A. W., Collier, A., Clarke, B.F. 1987. Secretor state of patients with type 1 or type 2 diabetes mellitus. British Medical Journal 295: 1024-1025.

24. Stuart, J.M., Cartwright, K.A.V., Jones, D.M., Noah, N.D., Wall, R.J., Blackwell, C.C., Jephcott, A.E. and Ferguson, I.R. 1987. An outbreak of meningococcal disease in Stonehouse: planning and execution of a large scale survey. Epidemiology and Infection 99: 579-589.

25. Blackwell, C.C., Jonsdottir, K., Mohammed, I. and Weir, D.M. Non-secretion of blood group antigens. A genetic factor predisposing to infection by Neisseria meningitidis in J.T. Poolman et al (eds). Gonococci and Meningococci. Kluwer Publishers, dordrecht 1988 pp. 633-636.

26. Collier, A., Patrick, A.W., Toft, A.D., Blackwell, C.C., James, V.S. and Weir, D.M. 1988. Increased prevalence of non-secretors in patients with Graves' disease - evidence for an infective aetiology? British Medical Journal 296: 1162.

27. Blackwell, C.C., Collier, A., Patrick, A.W., Weir, D.M. and Clarke, B.F. 1988. Secretor state and complement levels (C3 and C4) among patients with insulin dependent diabetes mellitus. Diabetes Research 9: 117-119.

28. Blackwell, C.C. 1989. Genetic susceptibility to infectious agents. (Leading Article). Proceedings of the Royal College of Physicians, Edinburgh, 19: 129-138.

29. Blackwell, C.C., Weir, D.M., James, V.S., Cartwright, K.A.V., Stuart, J.M. and Jones, D.M. 1989. The Stonehouse study: Secretor status and carriage of Neisseria meningitidis and Neisseria lactamica. Epidemiology and Infection 102: 1-10.

30. Blackwell, C.C., 1989. The role of ABO blood groups and secretor status in host defences. FEMS Microbiology Immunology 47: 341-50.

31. Blackwell, C.C. 1989. A.E. Mourant: A biographical note in celebration of his 85th birthday. FEMS Microbiology Immunology, 47: 315-316.

32. May, S.J., Blackwell, C.C., Brettle, R.P., MacCallum, C.J. and Weir, D.M. 1989. Non-secretion of ABO blood group antigens: a host factor predisposing to recurrent urinary tract infections and renal scarring. FEMS Microbiology Immunology 47: 383-388.

33. May, S.J., Rahat, A., Blackwell, C.C., MacCallum, C.J., Brettle, R.P. and Weir, D.M. 1989. Characterization of Escherichia coli strains evaluated from infected urine of secretor and non-secretor women. FEMS Microbiology Immunology 47: 377-382.

34. Thom, S.M., Blackwell, C.C., McCallum C.J., Weir, D.M., Brettle, R.P. Kinane, D.F. and Wray, D. 1989. Non-secretion of blood group antigens and susceptibility to infection by Candida species. FEMS Microbiology Immunology 47: 401-406.

35. May, S.J., Blackwell, C.C. and Weir, D.M 1989. Lewis^a blood group antigen as a receptor for Candida species. FEMS Microbiology Immunology 47: 407-410.

36. Blackwell, C.C., Jonsdottir, K., Hanson, M.F., Weir, D.M., Mohammed, I., Cartwright, K.A.V., Stewart, J.M. and Jones, D.M. 1989. Blood group,secretor status and susceptibility to bacterial meningitis. FEMS Microbiology Immunology 47: 351-356.

37. Blackwell, C.C., Aly, F.Z.M., James, V.S., Weir, D.M., Collier, A. Patrick, A.W., Cumming, C.G. and Clarke, B.F. 1989. Blood group, secretor status and oral carriage of Candida and other fungal species among patients with diabetes mellitus. Diabetes Research, 12: 101-104.

38. Blackwell, C.C., May, S.J., MacCallum, C.J., Brettle, R.P. and Weir, D.M. 1989. Secretor state and susceptibility to recurrent urinary tract infections pp 234-240. In Host-parasite interactions in urinary tract infections. [Ed. E.H. Kass and C. Svanborg-Eden].

39. Blackwell, C.C., Weir, D.M., James, V.S., Todd, W.T.A., Banatvala, N, Chaudhuri, A.K.R., Gray, H.G., Thomson, E.J. and Fallon, R.J. 1990. Secretor status, smoking and carriage of Neisseria meningitidis. Epidemiology and Infection 104: 203-209.

40. Blackwell, C.C. and Weir D.M. 1990. Meningococcal disease: high virulence and low tranmsission? Lancet 336: 53.

41. Toft, A.D., Blackwell, C.C., Saadi, A.T., Wu, P., Lymberi, P., Soudjidelli, M. and Weir D.M. 1990. Secretor status and infection in patients with Graves' disease. Autoimmunity, 7: 279-289.

42. Rahat, A., Stewart, J., Blackwell, C.C. and Weir, D.M. 1990. A semi-quantitative method for measurement of H type 1 and H type 2 on buccal cells and in saliva of secretors and non-secretors. Vox Sanguinis, 59: 101-105.

43. Mentis, A., Tzouvelekis, L., Spilliades, C, Blackwell, C.C. and Weir, D.M. 1990. Inhibition of Helicobacter pylori haemagglutination activity by human salivary mucins. FEMS Microbiol Immunol, 64: 125-128.

44. Mentis, A., Blackwell, C.C., Weir, D.M., Spiliades, G., Dailianas, A. and Skandalis, N. 1991. ABO blood group, secretor status and detection of Helicobacter pylori among patients with gastric or duodenal ulcers. Epidemiology and Infection 106: 221-229.

45. Aly, F.Z., Blackwell, C.C., Mackenzie, D.A.C., Weir D.M., Elton, R.A., Sofaer, J.A. and Clarke, B.F. (1991). Chronic atrophic oral candidiasis among patients with diabetes mellitus - the role of secretor status. Epidemiology and Infection 106: 355-363.

46. Raza, M.W., Blackwell, C.C., Molyneaux, P., James, V.S., Ogilvie, M.M., Inglis, J.M. and Weir, D.M. 1991. Association between secretor status and respiratory viral illness. British Medical Journal 303: 815-818.

47. Blackwell, C.C., James, V.S., Davidson, S., Wyld, R., Brettle, R.P., Robertson, R.J. and Weir D.M. 1991. Secretor status and heterosexual transmission of HIV. British Medical Journal 303: 825-826.

48. Tzouvelekis, L.S., Mentis, A.F., Makris, A.M., Spiliadis, C., Blackwell, C.C., and Weir, D.M. 1991. In vitro binding of Helicobacter pylori to human gastric mucin. Infection and Immunity 59: 4252-4254.

49. Blackwell, C.C., Tzanakaki, G., Kremastinou, J., Weir, D.M., Vakalis, N., Elton, R.A and Mentis, A 1992. Factors affecting carriage of Neisseria meningitidis among Greek military recruits. Epidemiology and Infection 108: 441-448.

50. Tzanakaki G, Blackwell CC, Kremastinou J, Kallergi C and Weir DM. Antibiotic sensitivities of meningococcal isolates obtained from patients and carriers in Greece. Epidemiology and Infection 108: 449-456.

51. Blackwell, C.C., Saadi, A.T., Raza, M.W., Stewart, J., Ogilvie, M.M., and Weir, D.M. Susceptibility to infection in relation to sudden infant death syndrome. Journal of Clinical Pathology 45 (supplement): 20-24.

52. Tzanakaki, G., Blackwell, C.C., Kremastinou, J., Kallergi, C., Fallon, R.J. and Weir, D.M. 1993. Serogroups, serotypes and subtypes of Neisseria meningitidis isolated from patients and carriers in Greece. Journal of Medical Microbiology 38: 19-22.

53. Zorgani, A.A., Stewart, J., Blackwell, C.C., Elton, R.A. and Weir, D.M. 1992. Secretor status and humoral immune responses to Neisseria lactamica and Neisseria meningitidis. Epidemiology and Infection 109: 445-452.

54. Aly, F.Z.M., Blackwell, C.C., Mackenzie, D.A.C., Weir, D.M. and Clarke, B.F. 1992. Factors influencing oral carriage of yeasts among individuals with diabetes mellitus. Epidemiology and Infection 109: 507-518.

55. Raza, M.W., Ogilvie, M.M., Blackwell, C.C., Stewart, J., Elton, R.A. and Weir, D.M. 1993. Effect of respiratory syncytial virus infection on binding of Neisseria meningitidis and Haemophilus influenzae type b to a human epithelial cell line (Hep-2). Epidemiology and Infection (in press).

56. Saadi, A.T., Blackwell, C.C., Raza, M.W., James, V.S., Stewart, J., Elton, R.A. and Weir, D.M. Factors enhancing adherence of toxigenic Staphylococcus aureus to epithelial cells and their possible role in sudden infant death syndrome. Epidemiology and Infection (in press).

57. Blackwell, C.C., Saada, A.T., Raza, M.W., Weir, D.M. and Busuttil, A. The potential role of bacterial toxins in sudden infant death syndrome (SIDS). International Journal of Legal Medicine (in press).

58. Kremastinou, J., Blackwell, C.C., Tzanakaki, G., Kallergi, C., Chronas, G., Elton, R.A., Mentis, A. and Weir, D.M. Parental smoking and carriage of Neisseria meningitidis among school children. Eighth International Pathogenic Neisseria Conference (in press).

59. Kremastinou, J., Tzanakaki, G., Mentis, A., Blackwell, C.C.and Weir, D.M. Carriage of Neisseria meningitidis among military recruits and school-age children in Greece. Eighth International Pathogenic Neisseria Conference (in press).

ref: ccb/mc/c/cv/ccb-dsc

SENSITIVITY OF *NEISSERIA GONORRHOEAE* TO PARTIALLY PURIFIED R-TYPE PYOCINES AND A POSSIBLE APPROACH TO EPIDEMIOLOGICAL TYPING

C. CAROLINE BLACKWELL, H. YOUNG AND ISABEL ANDERSON*

*Department of Bacteriology, Edinburgh University Medical School, Teviot Place, Edinburgh EH8 9AG, and *Westminster Medical School, Horseferry Road, London SW1P 2AR*

A RAPID, inexpensive typing system for *Neisseria gonorrhoeae* would be of use in epidemiological studies, for distinguishing failures of treatment from cases of reinfection, and in the early recognition of strains that might have special pathogenic features such as those associated with disseminated infection (Knapp and Holmes, 1975; Morello, Lerner and Bohnoff, 1976; Schoolnick, Buchanan and Holmes, 1976; Eisenstein, Lee and Sparling, 1977). Previous efforts to type gonococcal strains have involved the serological detection of antigenic differences (Glynn and Ward, 1970; Maeland, Kristoffersen and Hofstad, 1971; Apicella, 1974; Johnston, Holmes and Gotschlich, 1976; Tramont *et al.*, 1976; Wang *et al.*, 1977), differences in nutritional requirements (Carifo and Catlin, 1973) and variations in sensitivity to bacteriocines (Flynn and McEntegart, 1972; Walstad, Reitz and Sparling, 1974; Knapp, Falkow and Holmes, 1975; Lawton *et al.*, 1976).

Observations on the production of pyocines and demonstration of sensitivity to these bacteriocines have been used as the basis of typing systems for *Pseudomonas aeruginosa* (Farmer and Herman, 1969; Govan and Gillies, 1969). Sensitivity to R-type pyocines has been reported for strains of *N. gonorrhoeae* (Morse *et al.*, 1976) and the possible use of pyocines as a typing tool has been investigated (Sidberry and Sadoff, 1977). Three types of bacteriocines have been described in *Ps. aeruginosa:* soluble bacteriocines (S-type) similar to the colicines of *Escherichia coli* (Ito, Kageyama and Egami, 1970), rod-type (R-type) particles resembling defective bacteriophages (Kageyama, 1964), and the flexuous F-type bacteriocines (Govan, 1974*b*). The R-type and F-type pyocines were chosen for the present study because they can be sedimented by ultracentrifugation and are visible in electron micrographs; these qualities allow their partial purification and separation from the S-type pyocines and other soluble inhibitory substances produced by *Ps. aeruginosa.*

Using 23 pyocine preparations, Sidberry and Sadoff (1977) obtained 30 sensitivity patterns among 106 strains of *N. gonorrhoeae* but the majority of the strains fell into two groups and further evaluation of the method with gonococcal isolates from several sources was suggested by the authors.

Received 8 Dec. 1978; *accepted* 10 Jan. 1979

MATERIALS AND METHODS

Micro-organisms and culture media. Strains of *Ps. aeruginosa* producing the R-type pyocines listed in table I were obtained from Dr John Govan of this department. Kageyama (1975) classified many R-type pyocines on the basis of their spectrum of activity, and strains from groups R1, R2, R3 and R5 were specifically chosen for use in this study. The Kageyama classification for the R-type pyocines produced by these strains is given when known. Strains of *Ps. aeruginosa* from which F-type pyocines were obtained were pp430, F1, R186, Ep520, Ep111, R34, R7095, Ep274, Ru84, and Ep 397. Strains of *Pseudomonas* were maintained by subculture on nutrient agar every 7 days. *N. gonorrhoeae* strains were supplied by the Center for Disease Control (CDC), Atlanta, Ga, USA and the Royal Infirmary, Edinburgh (RIE). Organisms from disseminated infections were obtained from Dr Joan S. Knapp, Neisseria Reference Laboratory,

TABLE I

Pyocine-producing strains of Pseudomonas aeruginosa and their corresponding indicator strains

Kageyama classification	Pyocine-producing strain	Pyocine indicator strain
R1	IS4	ISC
	IS6	ISC
	IS8	ISC
	ISD	ISC
	2285	ISC, IS6
R2	ISB	ISC
	R21	IS8
	pp430	IS8
R3	ISE	IS4
R4	...	...
R5	ISC	IS4, IS5
Unclassified	R205	ISC

... = Not available for examination.

United States Public Health Service, Seattle, Washington; these included prototrophic strains and strains of different auxotypes from three geographic areas: Atlanta, Indianapolis, and Seattle. All RIE organisms had been identified as *N. gonorrhoeae* on the basis of Gram stain, oxidase reaction and sugar utilisation. On arrival at our laboratory each was retested for oxidase reaction and Gram staining. Strains were stored in skimmed milk at −20°C. The gonococcal strains were maintained on either Thayer Martin medium (TM) or modified New York City medium (MNYC; Young, 1978). When needed, colony types T1, T2, T3 and T4 were selected from GC Medium (Difco) with the aid of a stereoscopic microscope with a double system of substage lighting (Jephcott and Reyn, 1971).

Sensitivities to antibiotics were determined on clear GC Medium (Difco) with the following additions per 960 ml after autoclaving: 0·5% (w.v) ferric nitrate (British Drug Houses) 10 ml, GC supplement* 20 ml, 20% (w.v) glucose 10 ml. Antibiotics were added to GC agar at the following concentrations: penicillin G (Glaxo) sodium salt: 0·015, 0·03, 0·06, 0·125, 0·25, 0·5, 1, 2 units/ml; tetracyline hydrochloride (Achromycin, Lederle Ltd): 0·05, 0·1, 0·25, 0·5, 1 μg/ml; spectinomycin hydrochloride (Trobicin, Upjohn Ltd): 2, 4, 8, 16 μg/ml; chloramphenicol (Chloromycetin, Parke-Davis Ltd): 0·1, 0·2, 0·4, 0·8, 1·6 μg/ml; lincomycin hydrochloride (Upjohn Ltd): 4, 8, 16, 32 μg/ml.

* GC supplement: L glutamine (Koch Light Chemicals) 0·5 g, cocarboxylase (British Drug Houses) 0·01 g, water to 100 ml 20%.

Antibiotic-sensitivity testing. Visibly turbid suspensions (approximately 1×10^8–3×10^8 colony-forming units/ml) of gonococci were made in NaCl 0·85% (w/v) containing soluble starch 1% (starch-saline). Portions of the suspension were placed in the wells of a Steers replicator which delivered approximately 0·0025 ml of the suspension to the antibiotic plates (Steers, Foltz and Graves, 1959). The same suspensions were also used for the assay of sensitivity to pyocines. The plates were then incubated for 48 h at 37°C with 10% CO_2 in a Searle Qualitemp (80 TC) CO_2 incubator. Minimal inhibitory concentration (MIC) was recorded as the lowest concentration of antibiotic at which there were five or fewer colonies per spot inoculated.

Induction and preparation of pyocines. Pyocine preparations were obtained by a modification of the method of Govan (1974*a*). From an overnight nutrient-broth culture, 2 ml was added to 25 ml of sodium glutamate broth (SGB) which contained sodium glutamate 20 g, glucose (20% w/v solution) 20 ml, magnesium sulphate ($MgSO_4\ 7H_2O$) 0·1 g, disodium hydrogen phosphate ($Na_2HPO_4.\ 12H_2O$) 5·63 g, potassium dihydrogen phosphate (KH_2PO_4) 0·25 g, calcium nitrate 10 mg, yeast extract 1 g, and distilled water to 1 litre. The sterile glucose in solution (Seitz filtered) was added to the cooled autoclaved medium before use (Kageyama and Egami, 1962).

The pseudomonads were incubated at 30°C in an orbital incubator (Gallenkamp) at 100 r.p.m. for 2·5 h. Mitomycin C (1·5 μg/ml) was added at this point and the cultures were incubated for another 2·5–3 h. The lysates were centrifuged at 10 000 *g* for 30 min. to remove bacterial cells and debris; each supernatant was decanted, shaken with chloroform 5% (v/v) and stored at 4°C. This preparation was the crude pyocine lysate.

The crude lysate (12·5 ml) was ultracentrifuged at 100 000 *g* for 3 h and the supernatant containing S-type pyocine activity and other soluble inhibitory substances was discarded. The pellet containing the R-type activity was resuspended in 10 ml 0·01 M Tris buffer (*p*H 7·5) containing 0·01 M $MgCl_2 6H_2O$ and 0·01 M $MgSO_4 7H_2O$ (referred to hereafter as Tris buffer). This partially purified pyocine preparation was stored at 4°C.

Assay of pyocine activity against Ps. aeruginosa and N. gonorrhoeae. Pyocine activities of the crude lysates and the partially purified preparations were assayed by spotting doubling dilutions on nutrient-agar (NA) plates and MNYC plates and allowing the spots to dry. The NA plates were then flooded with the appropriate standard strains of *Ps. aeruginosa* (indicator strains) known to be sensitive to the particular test pyocine. Sensitive and resistant gonococcal strains, identified in pilot studies, were tested on MNYC.

Partially purified pyocine lysates were diluted 1 in 2 in Tris buffer and spotted with a dropper pipette calibrated to deliver 0·02 ml on plates of GC, MNYC or TM medium and allowed to dry. Overnight plate cultures of gonococci were suspended in starch-saline and used to flood the plates to produce a confluent lawn. The plates were allowed to dry and incubated overnight at 37°C aerobically with 10% CO_2. Some gonococci grew more slowly and were incubated for an additional 24 h. Results were recorded as + = inhibition, ± = inhibition with some overgrowth, − = no inhibition. *Ps. aeruginosa* indicator strains were included in each test as well as the gonococcal strains CDC486 or CDC046, which were sensitive to most pyocines tested, and strain 879 which was resistant to most pyocines tested. The sensitivity of a gonococcal strain to each pyocine was examined at least three times. Preparations were assayed regularly to monitor levels of active pyocines. Those with low titres usually gave equivocal or negative results. Table III includes representative data of pyocine titres and sensitivities of sensitive strains CDC486, CDC046 and resistant strain 879.

Serotyping of meningococci and sulphadiazine sensitivity testing were done by Dr R. J. Fallon, Department of Laboratory Medicine, Ruchill Hospital, Glasgow.

RESULTS

Preliminary studies

None of the F-type pyocines listed (see *Methods*) inhibited any of 84 gonococcal strains against which they were tested in pilot studies. Several of the R-type pyocines were able to inhibit 94·5% (104/110) of the gonococcal

strains selectively. Inhibition was observed on clear GC, TM, or MNYC or media; the darker background of the TM and MNYC plates facilitated readings and these media were less subject to contamination. Addition of 1% soluble starch or 2% bovine serum albumin to the GC plates did not affect inhibition of gonococci by the pyocines.

Comparison of sensitivities of different indicators to the test pyocines

Two types of zones of inhibition corresponding to those found for soluble (S-type) and rod-type (R-type) pyocines were observed with the gonococci. The S-type zones resembled those found with soluble bacteriocines (Fredericq, 1957) or other inhibitory substances that diffuse readily into agar away from the site of inoculation. Large zones of this type were observed with crude lysates. Typical R-type zones—smaller, discrete zones of inhibition limited to the site of inoculation (Ito *et al.*, 1970)—were found with the partially purified ultracentrifuged preparations. Table II illustrates the differences in inhibition produced by crude lysates and the partially purified pyocines. These were titrated against the appropriate *Pseudomonas* indicators and two gonococcal strains, one that had appeared to be resistant to pyocines tested in the pilot studies and one that appeared to be sensitive to many of the pyocines. Ultracentrifugation separated the heavier R-type particles from the S-type pyocines, pigments, and other soluble substances that had an inhibitory effect on the gonococci. The pyocines in table II were selected as examples of this effect because the partially purified pyocines of Kageyama groups R2 and R3 did not inhibit gonococci under the conditions used in the survey but the crude lysates did. These observations are of importance when our results are compared with those of other workers.

The concentration of the gonococcal inoculum usually made little difference to the inhibition observed with a series of twofold dilutions of pyocines up to 1 in 8192. Undiluted gonococcal inocula and tenfold dilutions up to 1 in 100

TABLE II

Titration of crude lysate and ultracentrifuged preparations against Ps. aeruginosa and N. gonorrhoeae

R-type pyocines from		Titre of the stated pyocine, preparation in tests with					
		Pseudomonas indicator		*sensitive* gonococci		resistant gonococci	
Kageyama group	strain	Crude pyocine	Ultracentri-fuged pyocine	Crude pyocine	Ultracentri-fuged pyocine	Crude pyocine	Ultracentri-fuged pyocine
R2	ISB	1024	128	128	1*	8	0*
	R21	8192	4096	128	4	8	0
	pp430	8192	8192	32	1	8	0
R3	ISE	8192	8192	32	1	8	0

* 1 = Undiluted preparation; 0 = no inhibition.

were tested. A more dilute suspension occasionally gave a clearer zone of inhibition; strains were retested with diluted suspensions when only faint reactions (±) were obtained with undiluted preparations. The partially purified pyocine preparations were diluted in twofold steps to 1 in 8192 and each dilution was spotted on to appropriate plates for assay of inhibitory activity against a known standard sensitive strain of *Ps. aeruginosa*, a sensitive gonococcal strain and a resistant gonococcal strain. The titres of inhibitory activity for the pyocines of Kageyama groups R1, R2, R3, and R5 and the pyocine that is unclassified are outlined in table III.

TABLE III

Comparative sensitivities of Ps. aeruginosa and N. gonorrhoeae to partially purified pyocines

R-type pyocines from		Titre of the partially purified pyocine preparation in tests with		
Kageyma group	strain	*Ps. aeruginosa* indicator	sensitive gonococci	resistant gonococci
R1	IS4	8192	1024	0
	IS6	8192	1024	0
	IS8	8192	1024	0
	2285	8192	1024	1
	ISD	1024	128	0
R2	ISB	128	0	0
	pp430	8192	1	0
	R21	4096	4	0
R3	ISE	8192	1	0
R4	...	...	...	...
R5	ISC	2048	1024	0
Unclassified	R205	8192	1025	0

Footnote as in table II.

Group R1. Preparations of pyocines in this group were potent in tests against the sensitive *Pseudomonas* indicator strain, and less potent against sensitive gonococci strain CDC486 or CDC046, usually by three dilution tubes. There was only a slight inhibition of the "resistant" strain 879 by the undiluted strain 2285 pyocine.

Group R2. Although the titres for pyocines pp430 and R21 on sensitive strains of *Ps. aeruginosa* were similar to those for pyocines for group R1, the sensitivity of gonococci to these pyocines was dramatically lower and not reliably reproducible. The titre for pyocine ISB determined with *Ps. aeruginosa* for this set of tests was lower than that of pp430 or R21 and no inhibition of gonococci was noted. Slight inhibition of the sensitive gonococcal strain was observed with another preparation of pyocine ISB that had a higher titre on the sensitive *Pseudomonas* indicator, but this was only with the undiluted pyocine.

Group R3. The only example of this group, pyocine ISE, yielded results similar to those observed with pyocines of group R2—high titres on tests against the sensitive *Ps. aeruginosa* indicator strain but little or no reproducible activity against gonococci tested.

Group R4. Our collection did not include a member of this group.

Group R5. Results obtained for pyocine ISC were similar to those found for pyocines of group R1—high titres on sensitive *Ps aeruginosa* strain and slightly lower titres for sensitive gonococci.

Unclassified. Pyocine R205 was not classifiable in any of the Kageyama groups. Its titres on sensitive strains of *Pseudomonas* and gonococci were similar to those found for groups R1 and R5.

At the higher dilutions of each pyocine, areas of inhibition of growth became increasingly turbid. Morse *et al.* (1976) made similar observations and suggested that this was evidence for a non-replicating inhibitory substance. Not all strains were sensitive at the same dilutions of the pyocines; therefore a dilution of 1 in 2 was chosen for the screening procedure. This dilution would provide a large number of pyocine particles and correspond to the dilution used by Sidberry and Sadoff (1977).

Typing of Neisseria spp. with partially purified pyocine preparations

Isolates from different sites of individual patients as well as isolates from known consorts were tested for sensitivity to pyocines and antibiotics. A typical set of results is presented in table IV. Sensitivities to penicillin G, lincomycin, tetracycline, chloramphenicol, and spectinomycin were determined for each isolate with the same culture suspension that was used for determining pyocine sensitivities. These "matched" isolates usually had very similar pyocine patterns and identical patterns of sensitivities to the above antibiotics. We observed no correlation between patterns of pyocine sensitivities and sensitivity to any particular antibiotic.

In table V pyocine-sensitivity patterns for CDC and RIE strains are compared. There was a broader distribution of strains than that found by Sidberry and Sadoff (1977). Although there were differences in numbers per pyocine type in the two groups, there were only five small patterns—F, G, I, J and M—that were found among the CDC isolates but not the RIE ones.

Table VI presents pyocine-sensitivity patterns for strains from disseminfections. There were no extraordinary sensitivities, e.g., to pyocines of Kageyama groups R2 or R3. Patterns of sensitivity were not associated with particular sites of isolation, penicillin sensitivity or auxotypes. The majority of strains from each of the three geographic regions had similar patterns.

Strains of *Neisseria meningitidis* from patients attending the Department of Venereology were also collected and several were tested for sensitivity to pyocines. Only two of those sent for typing were serotypable; strain 1042B was of serotype W135 and strain 1055A was of type Z′. The majority were serologically nontypable but were of different pyocine types. There were two patients from whom meningococci were isolated from genital sites. Strain 330B was

TABLE IV

Antibiotic and pyocine sensitivites of isolates from different sites of individual patients

Gonococcus isolate no.	Site	Inhibition by pyocines from producer strains											Minimal inhibitory concentration* of				
		ISD	2285	IS4	IS6	IS8	R205	ISE	R21	ISB	430	ISC	Pn	Lm	Cm	Tc	Sp
1045A	Urethra	–	–	–	–	–	+	–	–	–	–	+	0·015	> 32	0·8	0·1	8
B	Cervix	–	–	–	–	–	+	–	–	–	–	+	0·015	> 32	0·8	0·1	8
1047A	Urethra	+	–	+	+	–	+	–	–	–	–	+	0·015	> 32	0·8	0·1	16
B	Cervix	+	–	+	+	–	+	–	–	–	–	+	0·015	> 32	0·8	0·1	16
1048A	Urethra	+	–	+	+	+	+	–	–	–	–	+	0·015	> 32	0·8	0·1	16
B	Cervix	+	–	+	+	+	+	–	–	–	–	+	0·015	> 32	0·8	0·1	16
C	Rectum	+	–	+	+	+	+	–	–	–	–	+	0·015	> 32	0·8	0·1	16
1049A	Urethra	+	–	–	+	–	+	–	–	–	–	+	0·125	> 32	0·8	0·1	16
B	Cervix	+	–	–	+	–	+	–	–	–	–	+	0·125	> 32	0·8	0·1	16
C	Rectum	+	–	–	+	–	+	–	–	–	–	+	0·125	> 32	0·8	0·1	16
1051A	Urethra	+	–	+	+	–	+	–	–	–	–	+	0·015	> 32	0·8	0·1	16
B	Cervix	+	–	+	+	–	+	–	–	–	–	+	0·015	> 32	0·8	0·1	16
1063A	Urethra	+	+	+	+	–	+	–	–	–	–	+	0·015	> 32	0·4	0·5	16
B	Cervix	+	+	+	+	–	+	–	–	–	–	+	0·015	> 32	0·4	0·5	16
1078A	Urethra	+	+	–	+	–	+	–	–	–	–	+	0·015	> 32	0·4	0·25	16
B	Cervix	+	+	–	+	–	+	–	–	–	–	+	0·015	> 32	0·4	0·25	16

+ = Inhibition; – = no inhibition.

* Minimal inhibitory concentration = μg/ml except Pn = units/ml. Pn = penicillin G, Lm = lincomycin, Cm = chloramphenicol, Tc = tetracycline, Sp = spectinomycin.

TABLE V

Distribution of pyocine typing groups of N. gonorrhoeae

N. gonorrhoeae: pyocine-sensitivity pattern	Inhibition by pyocines from Kageyama group											Number of CDC strains (% of all CDC strains)	Number of RIE strains (% of all RIE strains)
	R1, strain					unclassified strain	R2, strain			R3, strain	R5, strain		
	ISD	2285	IS4	IS6	Is8	R205	ISB	R21	430	ISE	ISC		
A	−	−	−	−	−	−	−	−	−	−	−	3(4·8)	3(6·3)
B	−	−	−	−	−	−	−	−	−	−	+	5(8·1)	1(2·1)
C	−	−	−	−	−	+	−	−	−	−	+	1(1·6)	2(4·2)
D	+	−	−	−	−	+	−	−	−	−	+	1(1·6	1(2·1)
E	+	−	±	−	−	+	−	−	−	−	+	2(3·2)	11(22·9
F	+	+	−	−	−	+	−	−	−	−	+	1(1·6)	0(0)
G	+	−	+	+	−	−	−	−	−	−	+	3(4·8)	0(0)
H	+	−	+	+	−	+	−	−	−	−	+	8(12·9	7(14·6)
I	+	−	±	−	±	+	−	−	−	−	+	1(1·6)	0(0)
J	−	+	+	−	+	+	−	−	−	−	+	2(3·2)	0(0)
K	+	+	−	+	−	+	−	−	−	−	+	5(8·1)	5(8·1)
L	+	+	+	+	−	+	−	−	−	−	+	9(14·5)	6(12·5)
M	+	+	−	+	+	+	−	−	−	−	+	2(3·2)	0(0)
N	+	−	+	+	+	+	−	−	−	−	+	4(6·5)	10(20·8)
O	+	+	+	+	+	+	−	−	−	−	+	15(24·2)	2(4·2)
												Total 62	Total 48

− = No inhibition; + = inhibition; ± = inhibition with some overgrowth. CDC = Center for Disease Control; RIE = Royal Infirmary Edinburgh.

TABLE VI

Pyocine typing of gonococci from disseminated infections

Auxotype	Source	Strain number	Site of isolation	Penicillin MIC (units/ml)	Inhibition by pyocines from Kageyama group: R1, strain ISD	R1, strain 2285	R1, strain IS4	R1, strain IS6	R1, strain IS8	unclassified strain R205	R2, strain ISB	R2, strain R21	R2, strain 430	R3, strain ISE	R5, strain ISC
Protrophic	Seattle	1071	Cervix	0·5	+	+	+	+	+	+	−	−	−	−	+
	Indianapolis	6302	Joint	0·015	−	−	−	−	−	−	−	−	−	−	±
		6354	Cervix	0·125	−	−	−	−	−	−	−	−	−	−	±
	Atlanta	7422	Urethra	0·03	+	+	+	+	+	+	−	−	−	−	+
		7423	Blood	0·125	−	±	±	±	±	−	−	−	−	+	
pro⁻	Seattle	1947	Joint	1·0	+	+	+	+	+	+	−	−	−	−	+
	Indianapolis	6355	Cervix	0·015	−	−	−	−	−	−	−	−	−	−	+
	Atlanta	7416	Cervix	0·015	−	−	−	±	±	±	−	−	−	−	+
		7425	Blood	0·015	−	−	−	−	−	−	−	−	−	−	−
		7432	Blood	0·06	±	−	±	±	±	±	−	−	−	−	±
		7502	Blood	0·015	−	−	−	−	−	−	−	−	−	−	±
		7503	Joint	0·015	−	−	−	−	−	−	−	−	−	−	−
arg⁻	Atlanta	7406	Cervix	0·015	−	−	−	−	−	−	−	−	−	−	+
pro⁻, *arg*⁻, *hyx*⁻, *ura*−	Seattle	1567	Joint	0·015	+	−	−	−	−	−	−	−	−	−	+
arg⁻, *hyx*⁻, *ura*⁻	Seattle	867	Joint	0·015	−	−	−	−	−	−	−	−	−	−	+
		879	Cervix	0·015	−	−	−	−	−	−	−	−	−	−	−
		1241	Throat	0·015	+	−	±	±	+	+	−	−	−	−	+
		1255	Joint	0·015	+	−	+	+	+	+	−	−	−	−	+
		1341	Cervix	0·015	+	±	+	+	+	+	−	−	−	−	+
		1350	Urethra	0·015	+	−	±	±	±	±	−	−	−	−	+
		1362	Cervix	0·015	+	+	+	+	+	+	−	−	−	−	+
		1385	Blood	0·015	+	±	+	+	+	+	−	−	−	−	+
		1402	Blood	0·015	+	±	+	+	+	+	−	−	−	−	+
		1560	Blood	0·015	+	+	+	+	+	+	−	−	−	−	+

+ = Inhibition; − = no inhibition; ± = inhibition with some overgrowth.

TABLE VII

Sensitivity of N. meningitidis to R-type pyocines

Serotype	Strain number	Site	Sulphadiazine MIC (mg/litre)	Inhibition by pyocines from Kageyama group										
				R1, strain					unclassified strain	R2, strain			R3, strain	R5, strain
				ISD	2285	IS4	IS6	IS8	R205	ISB	R21	430	ISE	ISC
W135	1043B	Throat	5-10	−	−	−	−	−	−	−	−	−	−	−
Z′	1055A	Throat	1–5	+	−	−	−	−	+	−	−	−	−	+
Nontypable	339B	Cervix	1–5	+	−	−	−	+	+	−	−	−	−	+
	1056A	Throat	1–5	−	−	−	−	−	−	−	−	−	−	+
	1057A	Throat	0·1–1	+	−	+	+	+	+	−	−	−	−	+
	1058A	Throat	0·1–1	+	−	−	+	−	+	−	−	−	−	+
	1071A	Throat	0·1–1	+	−	−	−	−	+	−	−	−	−	+
	1093A	Throat	1–5	+	+	+	+	−	+	−	−	−	−	+
	1096A	Throat	1–5	+	+	+	+	−	+	−	−	−	−	+
	9102A	Cervix	5–10	±	±	−	−	±	±	−	−	−	−	+
	B	Urethra	1–5	±	±	±	±	±	±	−	−	−	−	+
	C	Rectum	1–5	±	±	±	±	±	±	−	−	−	−	+
	D	Throat	1–5	±	±	±	±	±	±	−	−	−	−	+

+ = Inhibition; − = no inhibition; ± = inhibition with some overgrowth.

found in a cervical culture and strains 9102 A, B, C, and D from cervix, urethra, rectum, and throat respectively. The genital isolates from these two patients were non-typable serologically and had different pyocine types. Three of the four isolates from patient 9102 had similar patterns of pyocine sensitivity and similar sensitivities to sulphadiazine (1–5 mg/ml). Strain 9102A was less sensitive to sulphadiazine (5–10 mg/ml) than the other three isolates and had a different pyocine-sensitive pattern (table VII). Further studies are in progress with a larger group of meningococci from different serological groups and sources.

Discussion

These results are additional evidence that tests for sensitivity of gonococci to R-type pyocines may provide a rapid, simple, inexpensive method for typing *N. gonorrhoeae* (Sidberry and Sadoff, 1977). Isolates from consorts or from different body sites of individual patients could generally be identified as being similar strains. Patterns of antibiotic sensitivities for these matched isolates agreed with the findings for pyocine sensitivities. Sensitivity to pyocines appears to be a stable characteristic. A fresh local isolate and a strain from a disseminated infection, both subcultured twice a week for over a year, have maintained their original patterns of sensitivity to the pyocines tested.

Preliminary results with organisms from several sources (CDC collection) and local isolates indicated that there is a broader distribution among the pyocine sensitivity types of gonococci observed than that found by Sidberry and Sadoff (1977) with their isolates from limited geographical locations. Using 23 pyocine lysates they found 30 types; but a majority of their strains 48% (51/106) fell into one group and 18% (19/106) into a second group. We found 15 types in tests with 11 pyocines and there are several more types to be defined with the additional pyocines being used in our present studies.

Table VIII presents an analysis of our results compared with those of Sidberry and Sadoff (1977). The analysis is based on the schematic representation of pyocine receptors in *Ps. aeruginosa* proposed by Kageyama (1975). It was observed in our study that if a gonococcal strain was sensitive to any of the pyocines, it was sensitive to ISC, a pyocine of Kageyama group R5; the gonococcal strains in both studies were most often sensitive to members of this group. Fewer strains were inhibited by pyocines of group R1 and the proportions of strains inhibited by this group of pyocines were similar in both studies. Our results differed in that we were unable to detect reproducible inhibition by R2 and R3 pyocines among the gonococcal strains tested. Crude lysates of R2 and R3 pyocines produced large S-type zones of inhibition on the "sensitive" (CDC486) and "resistant" (879) standard strains of gonococci, but the ultracentrifuged preparations did not inhibit either strain. Because the preparations used in Sidberry and Sadoff's studies were equivalent to our crude lysates, some of the inhibition by R2 and R3 pyocines that they observed with a few gonococcal strains may have been due to the presence of material other than the R-type pyocines.

TABLE VIII

Inhibition of N. gonorrhoeae by pyocines of Kageyama groups R1-R5; comparison of results with those of Sidbery and Sadoff (1977)

Percentage of *N. gonorrhoeae* isolates of indicated strain inhibited by pyocines of Kageyama group				
R3*	R4*	R2*	R1*	R5*
I = 9·4	H = 21·8	D = 0·9	K = 74	A = 100
		F = 2·8	L = 1·9	B = 100
		J = 16·4	M = 74·5	C = 100
		U = . . .	N = 76·8	E = 99·1
			O = 71·7	G = 99·1
			P = 61·9	
			Q = 5·7	
			R = 65·1	
			S = 66·9	
(Sidberry and Sadoff, 1977)			T = 61·4	
ISE = 0	. . .	ISB = 0	ISD = 84·5	ISC = 94·5
		R21 = 0	IS4 = 72·7	
		430 = 0	IS6 = 67·3	
			IS8 = 32·7	
(This study)			2285 = 42·7	

* Arranged in the order of the receptor sites on the lipopolysaccharide fraction of *Ps. aeruginosa* suggested by Kageyama (1975); the receptor for R5 is nearest to and that for R3 is furthest from the cytoplasm.

The receptor site for the R-type pyocines is in the lipopolysaccharide (LPS) (LPS) of *Pseudomonas* (Ikeda and Egami, 1973; Govan, 1974*a*; Koval and Meadow, 1977) and a similar location has been suggested for *Neisseria* (Sidberry and Sadoff, 1977). Table VIII is based on Kageyama's (1975) proposed pyocine receptor sites for his groups R1–R5 in the LPS of *Pseudomonas*. The receptor for R5 is in the portion of the LPS nearest the cytoplasm. The other receptors, in order towards the most distal portions of the LPS, are R1, R2, R4, R3. The "R" numbers correspond to sites thought to be essential for sensitivity to each pyocine; e.g., mutants lacking the receptor for R3 were resistant to pyocines of the R3 group but sensitive to those of groups R1, R2, R4, and R5. If the receptors for R-type pyocines in *Pseudomonas* and in gonococci are in the LPS, this analysis suggests a similarity in the "deeper" portions of the LPS of *Neisseria* and *Pseudomonas*. Other workers have also found that colony types T1 and T4 of the same strain showed no difference in sensitivity to the pyocine R-type 611, 131 (Morse *et al.*, 1976), indicating that pili probably do not form part of the receptor sites for the pyocines examined. The receptors for F-type pyocines are not found in the LPS fractions of *Pseudomonas* (Govan, 1974b) and, significantly, we did not find any inhibition of gonococci with the 10 F-type pyocines tested.

Gonococci from disseminated infections did not have unique pyocine sensitivities differing from those found for strains from localised infections.

The sensitivity of *Salmonella typhimurium* to some bacteriophages depends on the presence of receptor sites in the O repeat units of the LPS. Mutants that have lost these outer portions, rough or R-mutants, are less virulent than the smooth wild type. They are also insensitive to bacteriophages that attack the wild type because they lack the receptor (Rapin and Kalckar, 1971). If there were a unique portion in the outer LPS of the strains from disseminated gonococcal infection that resulted in serum resistance, or increased resistance to phagocytosis, it might be detectable with pyocines. Sensitivity was anticipated to pyocines of Kageyama groups R2 and/or R3, but no reproducible sensitivities were observed.

These findings are encouraging although further investigation with an emphasis on purification, standardisation and storage of the pyocine preparations used at present is clearly needed. Testing of other pyocines of the various Kageyama groups would be of value. Partially purified preparations of R2, R3 and R4 pyocines from other sources need to be examined to determine whether there are LPS receptors for these groups or whether the inhibitory effects reported by Sidberry and Sadoff (1977) were due to material other than the R-type pyocines. Pyocines that are "unclassified" by the Kageyama scheme may be useful because we have evidence for the selective inhibition of gonococci by pyocine R205 in the present study. In addition, selection of gonococcal indicator strains will be necessary.

Preliminary experiments indicate that this system may be able to differentiate strains of *Neisseria meningitidis* that are nongroupable by present serological methods.

Summary

Strains of *Neisseria gonorrhoeae* from a variety of sources were examined for sensitivity to 11 partially purified R-type pyocines from *Pseudomonas aeruginosa*. Selective inhibition of gonococci by pyocines of Kageyama groups R1 and R5 was observed. "Matched isolates", those from consorts or different body sites of individual patients, usually had very similar pyocine-sensitivity patterns and identical sensitivities to five antibiotics tested. This study included local isolates, strains from diverse geographic regions, and strains from disseminated gonococcal infections. It also proposed a relationship between pyocine-receptor sites in the lipopolysaccharide of *Ps. aeruginosa* and *N. gonorrhoeae*. Topics needing further evaluation are discussed.

We are grateful to Anne Lynch and Sheena Tuach for technical assistance and to Professor J. G. Collee and Dr J. R. W. Govan for advice in preparation of the manuscript. We also thank Professor B. P. Marmion for his encouragement and support of the project. This investigation was supported by grant K/MRS/50/C22 from the Biomedical Research Committee, Scottish Home and Health Department.

REFERENCES

Apicella, M. A. 1974. Antigenically distinct populations of *Neisseria gonorrhoeae:* isolation and characterization of the responsible determinants. *J. infect. Dis.*, **130**, 619.

CARIPO, K. AND CATLIN, B. W. 1973. *Neisseria gonorrhoeae* auxotyping: differentiation of clinical isolates based on growth responses on chemically defined media. *Appl. Microbiol.*, **26**, 223.

EISENSTEIN, B. I., LEE, T. J. AND SPARLING, P. F. 1977. Penicillin sensitivity and serum resistance are independent attributes of strains of *Neisseria gonorrhoeae* causing disseminated gonococcal infection. *Infect. Immun.*, **15**, 834.

FARMER, J. J. AND HERMAN, L. G. 1969. Epidemiological finger-printing of *Pseudomonas aeruginosa* by the production of and sensitivity to pyocin and bacteriophage. *Appl. Microbiol.*, **18**, 760.

FLYNN, J. AND MCENTEGART, M. G. 1972. Bacteriocins from *Neisseria gonorrhoeae* and their possible role in epidemiological studies. *J. clin. Path.*, **25**, 60.

FREDERICQ, P. 1957. Colicins. Ann. Rev. Microbiol. **11**, 7.

GLYNN, A. A. AND WARD, M. E. 1970. Nature and heterogeneity of the antigens of *Neisseria gonorrhoeae* involved in the serum bactericidal reaction. *Infect. Immun.*, **2**, 162.

GOVAN, J. R. W. 1974*a*. Studies on the pyocins of *Pseudomonas aeruginosa:* morphology and mode of action of contractile pyocins. *J. gen. Microbiol.*, **80**, 1.

GOVAN, J. R. W. 1974*b*. Studies on the pyocins of *Pseudomonas aeruginosa:* production of contractile and flexuous pyocins in *Pseudomonas aeruginosa*. *J. gen. Microbiol.*, **80**, 17.

GOVAN, J. R. W. AND GILLIES, R. R. 1969. Further studies in the pyocine typing of *Pseudomonas pyocyanea*. *J. med. Microbiol.*, **2**, 17.

IKEDA, K. AND EGAMI, F. 1973. Lipopolysaccharides of *Pseudomonas aeruginosa* with special reference to pyocin R receptor activity. *J. gen. appl. Microbiol., Tokyo*, **19**, 115.

ITO, S., KAGEYAMA, M. AND EGAMI, F. 1970. Isolation and characterication of pyocins from several strains of *Pseudomonas aeruginosa*. *J. gen. app. Microbiol., Tokyo*, **16**, 205.

JEPHCOTT, A. E. AND REYN, A. 1971. *Neisseria gonorrhoeae*. Colony variation I. *Acta path. microbiol. scand.*, B, **79**. 609.

JOHNSTON, K. H., HOLMES, K. K. AND GOTSCHLICH, E. C. 1976. The serological classification of *Neisseria gonorrhoeae*. 1. Isolation of the outer membrane complex responsible for serotypic specificity. *J. exp. Med.*, **143**, 741.

KAGEYAMA, M. 1964. Studies of a pyocin. I. Physical and chemical properties. *J. Biochem.*, **55**, 49.

KAGEYAMA, M. 1975. Bacteriocins and bacteriophages in *Pseudomonas aeruginosa*. *In* Microbial drug resistance, edited by S. Mitsuhashi and H. Hashimoto. *Tokyo*, p. 291.

KAGEYAMA, M. AND EGAMI, F. 1962. On the purification and some properties of a pyocin, a bacteriocin produced by Pseudomonas aeruginosa. *Life. Sci. Oxford*, **9**, 471.

KNAPP, J. S., FALKOW, S. AND HOLMES K. K. 1975. Re-evaluation of bacteriocinogeny in *Neisseria gonorrhoeae*. *J. clin. Path.*, **28**, 274.

KNAPP, J. S. AND HOLMES, K. K. 1975. Disseminated gonococcal infections caused by *Neisseria gonorrhoeae* with unique nutritional requirements. *J. infect. Dis.*, **132**, 204.

KOVAL, S. F. AND MEADOW, P. M. 1977. The isolation and characterization of lipopolysaccharide-defective mutants of *Pseudomonas aeruginosa* PAC 1. *J. gen. Microbiol.*, **98**, 387.

LAWTON, W. D., BELLINGER, M. A., SCHLING, M. AND GAAFAR, H. A. 1976. Bacteriocin production by *Neisseria gonorrhoeae*. *Antimicrob. Agents Chemother.*, **10**, 417.

MAELAND, J. A., KRISTOFFERSEN, T. AND HOFSTAD, T. 1971. Immunochemical investigations on *Neisseria gonorrhoeae* endotoxin. 2. Serological multispecificity and other properties of phenol-water preparations. *Acta path. microbiol. scand.*, B, **79**: 233.

MORELLO, J. A., LERNER, S. A. AND BOHNOFF, M. 1976. Characteristics of atypical *Neisseria gonorrhoeae* from disseminated and localized infections. *Infect. Immun.*, **13**, 1510.

MORSE, S. A., VAUGHAN, P., JOHNSON, D. AND IGLEWISKI, B. H. 1976. Inhibition of *Neisseria gonorrhoeae* by a bacteriocin from *Pseudomonas aeruginosa*. *Antimicrob. Agents Chemother.*, **10**, 354.

RAPIN, A. M. C. AND KALCKAR, H. 1971. The relation of bacteriophage attachment to lipopolysaccharide structure. *In* Microbiol Toxins, vol IV, edited by G. Weinbaum, S. Kadis, and S. J. Ajl, *New York*, p. 263.

SCHOOLNIK, G. K., BUCHANAN, T. M. AND HOLMES, K. K. 1976. Gonococci causing disseminated gonococcal infection are resistant to the bactericidal action of normal human sera. *J. clin. Invest.*, **58**, 1163.

SIDBERRY, H. D. AND SADOFF, J. C. 1977. Pyocin sensitivity of *Neisseria gonorrhoeae* and its feasibility as an epidemiological tool. *Infect. Immun.*, **15**, 628.

STEERS, E., FOLTZ, E. L. AND GRAVES, B. S. 1959. An inocula replicating apparatus for routine testing of bacterical susceptibility to antibiotics. *Antibiotics Chemother, 1954–1968*, **9**, 307.

TRAMONT, E. C., GRIFFISS, J. MCL., ROSE, D., BROOKS, G. F. AND ARTENSTEIN, M. S. 1976. Clinical correlation of strain differentiation of *Neisseria gonorrhoeae*. *J. infect. Dis.*, **134**, 128.

WALSTAD, D. L., REITZ, R. C. AND SPARLING, P. F. 1974. Growth inhibition among strains of *Neisseria gonorrhoeae* due to production of inhibitory free fatty acids and lysophosphatidylethanolamine: absence of bacteriocins. *Infect. Immun.*, **10**, 481.

WANG, S. P., HOLMES, K. K., KNAPP, J. S., OTT, S AND D. D. KYZER,, 1977. Immunological classification of *Neisseria gonorrhoeae* with microimmunofluorescence. *J. Immunol.*, **119**, 795.

YOUNG, H. 1978. Cultural diagnosis of gonorrhoea with modified New York City (MNYC) medium. *Br. J. vener. Dis.*, **54**, 36.

Journal of Infection (1981) 3, 370–378

Typing of non-serogroupable *Neisseria meningitidis* by means of sensitivity to R-type pyocines of *Pseudomonas aeruginosa*

C. Caroline Blackwell and Jacqueline A. Law

Department of Bacteriology, University of Edinburgh, Medical School, Teviot Place, Edinburgh EH8 9AG

Summary

Thirty-three (66 per cent) of 50 non-serogroupable isolates of *Neisseria meningitidis* could be typed on the basis of their sensitivities to partially purified rod-type (R-type) pyocines from 13 strains of *Pseudomonas aeruginosa*. Only seven (11 per cent) of 63 serogroupable meningococci were sensitive to the test pyocines. The pyocine typing method is particularly applicable to the labelling of strains in which autoagglutinability precludes the standard agglutination typing procedure.

Introduction

Our interest in non-typable strains of meningococci was stimulated by isolation of these organisms from the genitourinary tract and rectum of several patients attending the Department of Genitourinary Medicine, Royal Infirmary Edinburgh (Blackwell, Young and Bain, 1978).

Conventional serotyping of *Neisseria meningitidis* is based on bacterial agglutination tests that detect differences in meningococcal capsular polysaccharide antigens; nine major groups are recognised. Table I lists these

Table I *Serogroups of* N. meningitidis

Serogroup	Group specific polysaccharide
A	N-acetyl-3-0-acetyl mannosamine phosphate (α1–6)
B	N-acetyl neuraminic acid (α2–8)
C	N-acetyl and O-acetyl neuraminic acid (α2–9)
D	Not determined
W135	4-O-α-D-galactopyranosyl-β-D-N-acetyl neuraminic acid
X	N-acetyl glucosamine phosphate (α1–4)
Y	N-acetyl neuraminic acid: glucose
Z	1-O-2-acetamido-2-deoxy-α-D-galactopyranosyl-glycerol 3^1-phosphate
Z′ (29E)	3-deoxy-D-*manno*-octulosonic acid

0163–4453/81/040370+09 $01·00/0

groups and the chemical compositions of the specific polysaccharides that have been determined (Bhattacharjee, Jennings, Kenny, Martin and Smith, 1976; WHO, Technical Report Series, 1976; Jennings, Rosell and Kenny, 1979). There are, however, a number of meningococcal isolates that are not classifiable by this system, i.e. non-serogroupable strains. They are 'non-agglutinable', reactive with none of the specific group antisera; 'polyagglutinable', reactive with more than one specific group antiserum; or 'auto-agglutinable' in diluent in the absence of serum. Non-groupable meningococci that we isolated from the urethra and cervix of patients were found to be sensitive to partially purified rod-type pyocines produced by *Pseudomonas aeruginosa* (Blackwell, Young and Anderson, 1979). It seemed important to follow up this finding as a possible aid to epidemiological tracing.

Materials and methods

Standard serotypes, representative strains of each serogroup, and various non-groupable strains of *N. meningitidis* were obtained from the following sources: Bacteriology Laboratory, Royal Infirmary Edinburgh (RIE): Dr R. J. Fallon, Ruchill Hospital, Glasgow; Professor J.-P. Albert and Dr J. Etienne, Centre Collaborateur, O.M.S. de Reference et de Recherche pour les Meningocoques, Marseille-Armees, France. Dr W. D. Zollinger, Walter Reed Army Medical Center, Washington, D.C. kindly supplied us with meningococcal prototype LPS serotype strains. On receipt at our laboratory each was retested for oxidase reaction and Gram stain. Strains were stored in skimmed milk at −20°C or maintained on modified New York City Medium (MNYC) (Young, 1978). Strain RIE 1339 of *N. gonorrhoeae* and strain ZD9 39 of *Ps. aeruginosa* (from Dr M. Kageyama, Mitsubishi-Kasei Institute of Life Sciences, Tokyo) were used as positive controls for each test.

Strains of *Ps. aeruginosa* producing R-type pyocines listed in Table II were obtained from Dr John Govan of this department. Kageyama (1975)

Table II *Pyocine producing strains of* Ps. aeruginosa

Kageyama classification of R-type pyocines	Pyocine producing strains
R1	ISD, 2285, IS4, IS6, IS8
R2	ISB, R21, pp430
R3	ISE
R4	Not available
R5	ISA, ISC
Nongroupable	R205, 9579

classified a number of R-type pyocines on the basis of spectrum of activity and strains from his groups R1, R2, R3 and R5 were specifically chosen for this study. A representative of group R4 was not available at the time.

Preparations of R-type pyocines were induced and partially purified as described previously (Blackwell, Young and Anderson, 1979). Sensitivity of meningococci to the pyocines was determined using the same method employed for *N. gonorrhoeae* (Blackwell, Young and Anderson, 1979). Serogrouping and sulphadiazine sensitivity tests of meningococcal isolates from RIE were done by Dr R. J. Fallon.

Results

It was observed that if a meningococcal isolate was sensitive to any of the pyocines tested, it was usually sensitive to the pyocines of group R5 and the unclassified pyocine 9579. Those sensitive to pyocines of group R1 were also sensitive to those of groups R5 (ISA and ISC) and 9579. No meningococcal isolate was sensitive to pyocines of groups R2 and R3. Our gonococcal isolates had similar patterns of sensitivity to the classified pyocines (Blackwell, Young and Anderson, 1979) and also to 9579. Standard pyocine sensitive strains of *Ps. aeruginosa, N. gonorrhoeae* and *N. meningitidis* were tested in each experiment to monitor the activity of the pyocine preparations. As the pyocine preparations aged some of the − reactions became ± reactions. This could be correlated with a fall in titre of pyocine activity against the pseudomonas, gonococcal and meningococcal indicator strains.

Sensitivity to pyocines appears however to be a stable and reproducible property. A non-serogroupable strain (468) has been subcultured twice per week for over two years and it has maintained its original pattern of sensitivity to the pyocines tested. Over a six-week period, three other isolates with three different patterns of pyocine sensitivity were subcultured twice weekly and tested every two weeks. The patterns of pyocine sensitivity had not altered during this time. Storage of isolates in skimmed milk at −20°C for a year did not affect the pyocine sensitivity patterns.

Five groups of meningococcal isolates were examined:

(a) reference strains and clinical isolates of each serogroup;
(b) non-groupable isolates from Marseilles, Edinburgh and Glasgow;
(c) prototype LPS serotype strains;
(d) pharyngeal isolates of patients attending the sexually transmitted diseases clinic, Royal Infirmary, Edinburgh;
(e) genitourinary and rectal isolates from patients attending the sexually transmitted diseases clinic, Royal Infirmary, Edinburgh.

The reference strain for each serogroup and representatives of each group obtained from Dr Fallon and from our clinical laboratory were tested on at least three occasions for sensitivity to pyocines. Table III lists these

Table III *Numbers of serogroupable isolates sensitive to partially purified pyocines*

Serogroup	No. tested	No. sensitive to one or more pyocines
A	7	1
B	24	2*
C	10	1
W135	10	1
X	2	0
Y	5	0
Z'	4	1
Z	1	1†
Total	63	7

Percentage total sensitive = 11.

* Two of the Frasch serotype strains M978 and M982 showed sensitivity to some of the pyocines.

† The reference strain Z was the only available representative of this group.

reference strains, number of isolates of each serogroup tested, and the number of isolates sensitive to one or more pyocines. Seven (11 per cent) of the 63 serogroupable strains were reproducibly sensitive to one or more pyocines. Serogrouping of these isolates confirmed that they were expressing their group polysaccharide.

Table IV *Numbers of nongroupable isolates sensitive to partially purified pyocines*

Source	Number tested	Number sensitive to one or more pyocines	(%)
Marseilles	12	4	33·3
Edinburgh	14	13	92·8
Glasgow	24	16	66·7
Total	50	33	66*

* Percentage total sensitive.

Of the 50 non-groupable strains examined, 33 (66 per cent) were sensitive to one or more pyocines (Table IV). There was no apparent difference in sensitivity to the pyocines among the non-groupable, polyagglutinable or autoagglutinable strains. Table V shows the types of patterns observed and the distribution of these patterns among isolates from the three different sources: Glasgow, Edinburgh and Marseilles. Five of the patterns observed—A, B, L, N, O—are similar to those found for *N. gonorrhoeae* (Blackwell, Young and Anderson, 1979).

Table V *Distribution of pyocine typing groups among non-serogroupable isolates of* N. meningitidis *inhibition by pyocines from Kageyama groups*

	Kageyama group													Source					
	R1					unclassified		R3	R2			R5							
N. meningitidis Pyocine sensitivity pattern	ISD	2285	IS4	IS6	IS8	R205	9579	ISE	ISB	R21	430	ISA	ISC	Glasgow isolates	(%)	RIE isolates	(%)	Marseilles isolates	(%)
A*	−	−	−	−	−	−	−	−	−	−	−	−	−	8	33	1	7	8	67
B	−	−	−	−	−	−	+	−	−	−	−	+	+	5	21	1	7	3	25
	+	−	−	−	+	+	+	−	−	−	−	+	+	0	0	1	7	0	0
	+	−	−	+	+	+	+	−	−	−	−	+	+	0	0	1	7	0	0
L	+	±	+	+	−	+	+	−	−	−	−	+	+	0	0	2	14	0	0
N	+	−	+	+	+	+	+	−	−	−	−	+	+	6	25	5	36	1	8
O	+	±	+	+	+	+	+	−	−	−	−	+	+	5	21	3	21	0	0

* Letters correspond to similar patterns observed among isolates of *N. gonorrhoeae* (Blackwell, Young and Anderson, 1979).

Table VI *Pyocine sensitivities of LPS serotype strains*

		Kageyama group												
		R1					Unclassified		R3	R2			R5	
Serotype	Prototype strain (serogroup)	ISD	2295	IS4	IS6	IS8	R205	9579	ISE	ISB	R21	430	ISA	ISC
L1	1263 (C)	−	−	−	−	−	−	−	−	−	−	−	−	−
L2	35E (C)	−	−	−	−	−	−	±	−	−	−	−	±	±
L3	6275 (B)	−	−	−	−	−	−	−	−	−	−	−	−	−
L4	891 (C)	−	−	−	−	−	−	−	−	−	−	−	−	−
L5	M981 (B)	−	−	−	−	−	−	−	−	−	−	−	−	−
L6	M992 (B)	−	−	−	−	−	−	−	−	−	−	−	−	−
L7	6155 (B)	−	−	−	−	−	−	+	−	−	−	−	±	±
L8	M978 (B)	+	+	−	+	+	+	+	−	−	−	−	+	+
L9	120M (A)	−	−	−	−	−	−	+	−	−	−	−	±	±
L10	7880 (A)	−	−	−	−	−	−	+	−	−	−	−	±	±

+ = inhibition; ± = inhibition with some overgrowth; − = no inhibition.

Pyocine sensitivities of meningococcal strains with different LPS compositions obtained from Dr W. D. Zollinger are shown in Table VI. Serotypes L1, L3, L4, L5 and L6 were insensitive to the pyocines tested. Serotypes L2, L7, L9 and L10 had similar sensitivities to pyocines ISA, ISC and 9579. Strain M978, serotype L8, was sensitive to pyocines of groups R1 and R5 and also the two unclassified pyocines R205 and 9579.

Table VII *Pyocine sensitivities of* N. meningitidis *from pharyngeal cultures*

	Kageyama group											
	R1					Unclassified		R3	R2			R5
No. of isolates	ISD	2285	IS4	IS6	IS8	R205	9579	ISE	ISB	B21	430	ISC
11	−	−	−	−	−	−	−	−	−	−	−	−
6	−	−	−	−	−	−	−	−	−	−	−	+
2	−	−	−	−	−	−	+	−	−	−	−	−
1	−	−	−	−	−	−	+	−	−	−	−	+
1	+	−	−	−	−	+	+	−	−	−	−	+
1	+	−	−	−	−	+	−	−	−	−	−	+
1	+	−	±	±	−	+	+	−	−	−	−	+
2	+	−	−	+	−	+	+	−	−	−	−	+
1	+	−	+	+	+	+	+	−	−	−	−	−
3	+	+	+	+	−	+	+	−	−	−	−	+
1	+	+	+	+	+	+	+	−	−	−	−	+

+ = inhibition; ± = inhibition with some overgrowth; − = no inhibition.

A group of 30 pharyngeal isolates was then tested for sensitivity to pyocines. Eleven (36·7 per cent) were resistant to all the pyocines tested. Six of the 19 isolates showed a single pattern and the remainder fell into 1(groups (Table VII). Seven of the 30 isolates, each with a different pattern of sensitivity to the pyocines tested, were sent for serogrouping. Five were non-groupable, one was group Z and one was group W135. The W135 strain was resistant to each of the pyocines tested.

Table VIII *Pyocine sensitivities of* N. meningitidis *from rectal or genitourinary cultures*

					Kageyama group									
					R1					R2		R3		R5
Patient	Serotype	Sulfadiazine sensitivity mg/ml	Site	Pyocin no.	ISD	2285	IS4	IS6	IS8	ISB	R21	430	ISE	IS(
A	NG	0·1–1	cervix	+	−	−	−	+	−	−	−	−	−	+
B	NG	. . .	urethra	−	−	−	−	−	−	−	−	−	−	+
C1	NG	1–5	urethra	+	+	+	+	+	−	−	−	−	−	+
C2	NG	5–10	cervix	+	−	−	+	+	−	−	−	−	−	+
C3	NG	1–5	rectum	+	+	+	+	+	−	−	−	−	−	+
C4	NG	1–5	throat	+	+	+	+	+	−	−	−	−	−	+
D	NG	1–5	urethra	+	−	+	+	+	−	−	−	−	−	+
E	NG	. . .	urethra	+	−	+	+	+	−	−	−	−	−	+
F	NG	. . .	urethra	+	−	+	+	+	−	−	−	−	−	+

. . . = not done; + = inhibition; ± = inhibition with some overgrowth; − = no inhibition.

Isolates of *N. meningitidis* cultured from throat, urethra, rectum or cervix of patients attending the Department of Venereology, Royal Infirmary, Edinburgh were examined for serogroup, sulphadiazine sensitive and sensitivity to pyocines. In Table VIII these data for isolates from six patients are compared. Although the meningococcal isolates were non-serogroupable, they could be separated into groups based on sensitivity to pyocines. Among the isolates from patient C, those from the urethra, rectum and throat had similar pyocine sensitivities and similar sensitivities to sulphadiazine. The cervical isolate differed in its pyocine sensitivity pattern and it was also more resistant than the other three to sulphadiazine.

Discussion

Sensitivity of *Neisseria gonorrhoea* to pyocines is thought to depend on the presence of specific receptor sites within the lipopolysaccharide (LPS) (Sidberry and Sadoff, 1977; Blackwell, Anderson and Young, 1979). A number of investigations have reported similarities between the LPS

structures of gonococci and meningococci (Tramont, Sadoff and Artenstein, 1974; Tramont, Griffiss, Rose, Brooks and Artenstein, 1976; Sadoff, Zollinger and Sidberry, 1978). Since there is also evidence for differences in the LPS components of different strains of meningococci (Davis and Arnold, 1974; Zollinger, Pennington and Artenstein, 1977; Mandrell and Zollinger, 1977), we extended our investigation on pyocine typing of *N. gonorrhoeae* to include a number of strains of *N. meningitidis* that were not serogroupable by their capsular antigens.

Among the 50 non-groupable isolates of *N. meningitidis* tested for sensitivity to 12 selected pyocines, 33 (66 per cent) were sensitive to one or more of the pyocines tested. As with the gonococcal strains tested, meningococci sensitive to any of the pyocines were sensitive to those of group R5 and the unclassified 9579. No strains were sensitive to pyocines of group R1 only. None of our isolates were sensitive to pyocines of groups R2 and R3 (Blackwell, Young and Anderson, 1979). The same patterns of sensitivity were observed for serogroupable isolates but only 11 per cent of these isolates were sensitive to one or more pyocines. The presence of the polysaccharide capsule may prevent the R-type pyocines from reaching the attachment site within the LPS. In Table III it is noteworthy that three of the seven serogroupable strains sensitive to pyocines (one from group A, one from group C, and one from group Z') were not laboratory strains. The other serotypable, pyocine sensitive strains were reference Z, reference W135, and two Frasch serotype strains (Table III) and the LPS prototype strains, 35E, 6155, 6275, M978 and 7880 (Table VI). The effect long term subculture has on maintenance of the capsule is an important consideration as this might affect the subsequent response to pyocines.

Only 5 per cent of gonococci tested in a previous study were insensitive to all pyocines used (Blackwell, Young and Anderson 1979). A portion of the 34 per cent of non serogroupable meningococcal strains insensitive to pyocines may possess capsular material that blocks the pyocine receptors in the LPS. New serogroups might be determined by investigating the pyocine insensitive strains.

Determination of pyocine sensitivities for non-serogroupable strains of *N. meningitidis* may be of value as an epidemiological tool in that the majority (66 per cent) of the total number tested are sensitive and may be typed by this method (Table IV). Three general patterns are apparent: (1) those that are insensitive to pyocines tested, (2) those that are sensitive to pyocines 9579, ISC and ISA, (3) those that are sensitive to pyocines 9579, ISA, ISC and pyocines of group R1 (Table V). Five of the seven patterns of sensitivity are similar to ones found with *N. gonorrhoeae* (Blackwell, Young and Anderson, 1979). Since about 50 per cent of non serogroupable isolates examined by one laboratory are autoagglutinable (R. J. Fallon, personal communication), the pyocine sensitivity method might provide a useful method for typing these strains even if new serogroups based on agglutination of capsular antigens are determined.

(We are grateful to Sheena Tuach for technical assistance, Dr Hugh Young for help in collection of *N. meningitidis* isolates from RIE and to Professor J. G. Collee and Dr D. M. Weir for advice in preparation of the manuscript. This investigation was supported by Grant K/MRS/50/C22 from The Biomedical Research Committee, Scottish Home and Health Department.)

References

Bhattacharjee, A. K., Jennings, H. J., Kenny, C. P., Martin, A. and Smith, I. C. P. (1976). Structural determination of the polysaccharide antigens of *N. meningitidis* serogroups Y, W135 and B01. *Canadian Journal of Biochemistry*, **54**, 1.

Blackwell, C. C., Young, H. and Anderson, I. (1979). Sensitivity of *Neisseria gonorrhoeae* to partially purified R-type pyocines and a possible approach to epidemiological typing. *Journal of Medical Microbiology*, **12**, 321.

Blackwell, C. C., Young, H., and Bain, S. S. R. (1978). Isolation of *Neisseria meningitidis* and *Neisseria catarrhalis* from the genitourinary tract and anal canal. *British Journal of Venereal Diseases*, **54**, 41.

Cerebrospinal Meningitis Control. Technical Report Series 588. World Health Organization, Geneva, (1976) pp. 6–7.

Davis, C. E. and Arnold, K. (1974). Role of meningococcal endotoxin in meningococcal purpura. *Journal of Experimental Medicine*, **140**, 159.

Jennings, H. J., Rosell, K.-G. and Kenny, C. P. (1979). Structural elucidation of the capsular polysaccharide antigen of *N. meningitidis* serogroup Z using ^{13}C nuclear magnetic resonance. *Canadian Journal of Chemistry*, **57**, 2902.

Kageyama, M. In *Microbial Drug Resistance*. Report of the International Symposium on Bacterial Resistance. (Mitsuhashi, S. and Hashimoto, H. Eds). University of Tokyo (1975) pp. 291–305.

Mandrell, R. E. and Zollinger, W. D. (1977). Lipopolysaccharide serotyping of *Neisseria meningitidis* by haemagglutination inhibition. Infection and Immunity, **16**, 471.

Sadoff, J. C., Zollinger, W. D. and Sidberry, H. *Cell surface structures of Neisseria gonorrhoeae. Immunobiology of Neisseria gonorrhoeae*. American Society for Microbiology Washington, D.C. (1978). pp. 93–100.

Sidberry, H. D. and Sadoff, J. C. (1977). Pyocin sensitivity of *Neisseria gonorrhoeae* and its feasibility as an epidemiological tool. *Infection and Immunity* **15**, 628.

Tramont, E. C., Griffiss, J. M., Rose, D., Brooks, G. F. and Artenstein, M. S. (1976). Strain differentiation of *Neisseria gonorrhoeae*. *Journal of Infectious Diseases*, **134**, 128.

Young, H. (1978). Cultural diagnosis of gonorrhoea with modified New York City (MNYC) medium. *British Journal of Venereal Diseases*, **54**, 36.

Zollinger, W. D., Pennington, C. L. and Artenstein, M. S. (1974). Human antibody response to three meningococcal outer membrane antigens: comparison by specific haemagglutination assays. *Infection and Immunity*, **10**, 975.

J. MED. MICROBIOL.—VOL. 15 (1982) 247–251

0022-2615/82/0502 0247 $02.00

SENSITIVITY OF THERMOPHILIC CAMPYLOBACTERS TO R-TYPE PYOCINES OF *PSEUDOMONAS AERUGINOSA*

C. CAROLINE BLACKWELL, F. P. WINSTANLEY AND W. A. TELFER BRUNTON

Department of Bacteriology, Edinburgh University Medical School, Teviot Place, Edinburgh EH8 9AG

SUMMARY. Strains of thermophilic campylobacters of human origin were examined for bacteriocine activity and for susceptibility to R-type pyocines of *Pseudomonas aeruginosa*. None of 50 strains inhibited the growth of any other strain, but 13 of 80 strains (16%) were sensitive to R-type pyocines. Absorption of one of the partially purified pyocine preparations with a sensitive strain resulted in the removal of pyocine activity and a decrease in viable count of the organism by 85%.

INTRODUCTION

The importance of campylobacters as animal pathogens has been recognised for many years. Human campylobacter infection was first reported by Vinzent, Dumas and Picard (1947), but during the next decade only 15 cases were reported (King, 1957). More recently, the thermophilic members of the genus have been shown to be commonly associated with diarrhoeal disease in man (Butzler *et al.*, 1973; Skirrow, 1977; Telfer Brunton and Heggie, 1977). Large outbreaks have been attributed to ingestion of contaminated water (Trehan and Vogt, 1978) and contaminated milk (Robinson *et al.*, 1979).

Studies of the epidemiology of campylobacter enteritis in man have been hindered by difficulties in classification and by the lack of simple methods for typing the causative organism. Biotyping has provided useful information but is time consuming and exacting (Skirrow and Benjamin, 1980). The development of serological methods has been hindered by difficulties in detecting somatic antigens (Butzler and Skirrow, 1979; Watson, Kerr and McFadzean, 1979), but they have now proved their value in epidemiology (Jones, Eldridge and Dale, 1980) and taxonomy (Penner and Hennessy, 1980). We have not found any reports of successful typing by means of bacteriocines or bacteriophages in the literature.

In this study we examined two possible methods for typing thermophilic campylobacters: examining strains for bacteriocine activity, and testing their sensitivity to partially purified preparations of rod-type (R-type) pyocines of *Pseudomonas aeruginosa*, which are of use for typing *Neisseria gonorrhoeae* (Blackwell, Young and Anderson, 1979), and non-serogroupable strains of *N. meningitidis* (Blackwell and Law, 1981).

MATERIALS AND METHODS

Bacteria. Thermophilic strains of *Campylobacter* were isolated from the faeces of patients or animals by the method of Skirrow (1977) and had the following characters: positive oxidase and catalase reactions; sensitive to nalidixic acid; growth at 43°C but not at 25°C. In all, 80 strains, each representing a separate isolation, were used in the following experiments. They were grown in an atmosphere containing *c.* 5% O_2 and 5% CO_2 (Skirrow, 1977).

Strains of *P. aeruginosa* that produce R-type pyocines, listed in table I, were obtained from Dr J. R. W. Govan of this department and Dr M. Kageyama, Mitsubishi-Kasei Institute of Life Sciences, Tokyo. Kageyama (1975) classified many R-type pyocines into groups on the basis of their spectra of activity; strains representing each of these groups were chosen for this study. *P.*

Received 26 May 1981; *accepted* 26 Aug. 1981.

aeruginosa strain ZD8/38, also from Dr Kageyama and sensitive to all the pyocines tested, was used as a positive indicator strain.

Screening of campylobacter strains for bacteriocine activity. Fifty strains of thermophilic campylobacters were tested against one another for the production of bacteriocines by the method of Gillies and Govan (1966). "Producer strains" were grown overnight (18 h) at 37°C on Tryptone Soya Agar (Oxoid) supplemented with 5% (v/v) of defibrinated horse blood. "Indicator strains" were grown overnight (18 h) in nutrient broth (Oxoid Nutrient Broth No. 2). This experiment was repeated with producer strains (1) that had been grown overnight on tryptone soya agar at 35°C, and (2) log-phase cultures of which had been grown for 18 h at 37°C in nutrient broth containing mitomycin C (1 μg/ml) before being plated on to tryptone soya agar and grown for 18 h at 37°C. Areas of inhibition of growth of the indicator strains by producer strains were taken as evidence of bacteriocine-like activity.

Assay of pyocine activity against campylobacter strains. The R-type pyocines were induced and partially purified as described previously (Blackwell *et al.*, 1979), after the method of Govan (1974). Sensitivity to R-type pyocines was determined by placing drops (15 μl) of a 1 in 2 dilution of each pyocine preparations on to plates of (1) tryptone soya agar supplemented with 5% (v/v) of defibrinated horse blood, (2) nutrient agar (Columbia Agar Base, Oxoid) containing agar 1% (w/v), and (3) similar nutrient agar containing agar 2% (w/v). After the drops had been absorbed into the agar, the plates were flooded with a 1 in 100 dilution of an overnight culture of a campylobacter strain in nutrient broth. Excess inoculum was removed by means of a sterile pipette, and the plates were then incubated overnight at 37°C in microaerobic conditions as described above. Results were recorded as + = inhibition, ± = inhibition with some overgrowth, − = no inhibition.

Preparation of electronmicrographs. A negative-staining technique described by Govan (1974) was used.

Titration of pyocine activity. Twofold dilutions of the pyocine suspension were made in Dulbecco's Buffered Salt Solution (BSS; Dulbecco A, Oxoid) with 0·2% phenol red. A drop (15 μl) of each dilution was placed on a dried plate of nutrient agar (agar 2% w/v). When the drops had been absorbed the plate was flooded with a suspension of *P. aeruginosa* strain ZD8/38 (10^7 cfu/ml), and incubated at 37°C for 18 h. The pyocine titre was the highest dilution that produced a clear area of inhibition of the bacterial lawn.

Adsorption of pyocine activity by campylobacter cultures. A turbid suspension of the campylobacter containing 10^8–10^9 cfu/ml was made in BSS. Equal volumes of bacterial suspension and pyocine preparation were mixed, incubated at 37°C for 30 min and then

TABLE I

Pyocine-producing strains of Pseudomonas aeruginosa

Kageyama classification	Number of pyocine-producing strain
R1	IS4 IS6 IS8 ISD 2285
R2	ISB R21 pp-430
R3	ISE
R4	R4
R5	ISC ISA
Unclassified	R205 9579

centrifuged for 15 min at 5000 rpm. The supernatant fluid was titrated for pyocine activity as described above. The pellet was resuspended to the original volume in BSS and the viable count determined by the method of Miles, Misra and Irwin (1938).

Results

Production of bacteriocines by campylobacter strains

None of the 50 strains inhibited the growth of any other strain, whether it had been grown at 37°C or 35°C or pre-incubated with mitomycin C. Thus, none of them gave evidence of bacteriocine-like activity in the conditions examined.

Sensitivity of campylobacter strains to R-type pyocines

Of the 80 strains examined, 13 (16%) were sensitive to one or more of the pyocine preparations. These were most often and most markedly sensitive to pyocines of strains 9579, ISA and ISC. Two isolates were sensitive to these three and also to R205 (unclassified) and three pyocines of group R1: ISD, IS6 and IS8. Sensitivity to any of the pyocines of groups R2, R3 or R4 was not observed (table II).

In addition to the initial sensitivity tests on blood agar, the experiments were repeated on 1% and 2% nutrient agar on which growth tended to be less mucoid, so that extracellular material would be less likely to interfere with the attachment of pyocines. Identical results were obtained on all three media.

Electronmicrographic studies

We were unable to demonstrate attachment of pyocine no. 9579 to campylobacter strains that were sensitive to its inhibitory activity.

Adsorption of pyocine activity by campylobacter strains

Absorption studies were performed with a preparation of pyocine no. 9579 (titre 256) and a pyocine-sensitive campylobacter strain (no. 13624) and a pyocine-resistant campylobacter strain (no. 01/T). After absorption with the resistant strain the titre of pyocine was 128 and after absorption with the sensitive strain it was less than 4. The viable count of the two suspensions used to adsorb the pyocine were determined before adding the pyocine suspension and at the end of the experiment. There was no decrease in the viable count of the resistant isolate during incubation with the pyocine but the viable count of the sensitive isolate was reduced by 85%, i.e., from $1{\cdot}51 \times 10^8$ cfu/ml to $2{\cdot}25 \times 10^7$ cfu/ml.

Table II

Inhibition of campylobacter strains by extracts containing R-type pyocines

Number of campylobacter strains	Inhibition of the stated number of campylobacter strains* by pyocines of Kageyama group													
	R1					R2			R3	R4	R5		Unclassified	
	ISD	2285	IS4	IS6	IS8	ISB	R21	pp-430	ISE	R4	ISA	ISC	R205	9579
67	−	−	−	−	−	−	−	−	−	−	−	−	−	−
4	−	−	−	−	−	−	−	−	−	−	−	−	−	−
2	−	−	−	−	−	−	−	−	−	−	+	−	−	+
5	−	−	−	−	−	−	−	−	−	−	+	+	−	−
2	±	−	−	±	±	−	−	−	−	−	+	±	±	±

+ = Inhibition of growth; ± = partial inhibition of growth; − = no inhibition of growth.
* Reference number of pyocine-producing strain (see table I).

Discussion

Sensitivity to R-type pyocines has been found to be of value in typing strains of *P. aeruginosa* (Gillies and Govan, 1966), strains of *N. gonorrhoeae* (Blackwell *et al.*, 1979) and non-serogroupable *N. meningitidis* (Blackwell and Law, 1981). As far as we are aware, the sensitivity of thermophilic campylobacters to R-type pyocines has not been reported previously. We were unable to demonstrate the attachment of pyocines to campylobacter cells by electronmicroscopy, possibly because of the instability of the campylobacter cell envelope, but we were able to remove the pyocine activity by absorption with suspensions of pyocine-sensitive campylobacters but not of pyocine-resistant campylobacters.

Only a small minority of the cultures tested were sensitive to the pyocines used in this study. Similar results were obtained with capsulate serogroupable meningococcal strains (Blackwell and Law, 1981). The capsular material of campylobacter strains may prevent the pyocines from penetrating to the receptor sites. Among 13 campylobacter isolates sensitive to the pyocines there were four distinct patterns of sensitivity (table II).

The receptor site for R-type pyocines is in the lipopolysaccharide (LPS) of the cell envelope of *P. aeruginosa* (Ikeda and Egami, 1973; Govan, 1974; Koval and Meadow, 1977) and a similar location has been suggested in *Neisseria* (Sidberry and Sadoff, 1977; Sadoff, Zollinger and Sidberry, 1978; Blackwell *et al.*, 1979). Because campylobacter cell walls closely resemble those of other gram-negative organisms, similar receptors for R-type pyocines may exist in the LPS of campylobacters. Our results suggest that some campylobacter strains share similar cell-envelope components, and sensitivity to the various groups of pyocines may reflect this.

Sensitivity to pyocines occurs too infrequently among thermophilic campylobacters to form the basis of an independent typing scheme, but it might be of help in the further development of serological typing schemes, in that it may provide a means of detecting differences in somatic antigens. The different LPS types might be used to develop assay systems similar to the microbactericidal assay used to classify *N. meningitidis*, group B (Frasch and Chapman, 1972), or the haemagglutination inhibition (Mandrell and Zollinger, 1977) and radioimmunoassay methods (Zollinger and Mandrell, 1977) used to determine LPS serotypes among capsulate strains of meningococci.

We are grateful to Mrs Marilyn Cole and Mrs J. Mears for help in typing the manuscript and to Professor J. G. Collee and Dr D. M. Weir for their advice in preparation of the manuscript. This investigation was supported by grant no. K/MRS/50/C22 from the Biomedical Research Committee, Scottish Home and Health Department, and Leo Laboratories Ltd.

REFERENCES

Blackwell, C. C. and Law, J. A. 1981. Sensitivity of *Neisseria meningitidis* to R-type pyocines of *Pseudomonas aeruginosa* and its possible use in typing non-serogroupable strains. *J. Infect.*, **3**, 370.

Blackwell, C. C., Young, H. and Anderson, I. 1979. Sensitivity of *Neisseria gonorrhoeae* to partially purified R-type pyocines and a possible approach to epidemiological typing. *J. med. Microbiol.*, **12**, 321.

Butzler, J. P., Dekeyser, P., Detrain, M. and Dehaen, F. 1973. Related vibrio in stools. *J. Pediat.*, **82**, 493.

Butzler, J. P. and Skirrow, M. B. 1979. Campylobacter enteritis. *In* Clinics in gastroenterology, 8 (3), edited by H. P. Lambert, W. B. Saunders Co. Ltd., London, p. 737.

Frasch, C. E. and Chapman, S. S. 1972. Classification of *Neisseria meningitidis* group B into distinct serotypes. 1. Serological typing by a microbactericidal method. *Infect. Immun.*, **5**, 98.

Gillies, R. R. and Govan, J. R. W. 1966. Typing of *Pseudomonas pyocyanea* by pyocine production. *J. Path. Bact.*, **91**, 339.

Govan, J. R. W. 1974. Studies on the pyocins of *Pseudomonas aeruginosa*: morphology and mode of action of contractile pyocins. *J. gen. Microbiol.*, **80**, 1.

Ikeda, K. and Egami, F. 1973. Lipopolysaccharides of *Pseudomonas aeruginosa* with special reference to pyocin R receptor activity. *J. gen. appl. Microbiol.*, **19**, 115.

JONES, D. M., ELDRIDGE, J. AND DALE, B. 1980. Serological response to *Campylobacter jejuni/coli* infection. *J. clin. Path.*, **33**, 767.

KAGEYAMA, M. 1975. Bacteriocins and bacteriophages in *Pseudomonas aeruginosa*. *In* Microbial drug resistance. Report of the International Symposium on Bacterial Resistance, edited by S. Mitsuhasi and H. Hashimoto, University of Tokyo Press, p. 291.

KING, E. O. 1957. Human infections with *Vibrio fetus* and a closely related vibrio. *J. infect. Dis.*, **101**, 119.

KOVAL, S. F. AND MEADOW, P. M. 1977. The isolation and characterization of lipopolysaccharide-defective mutants of *Pseudomonas aeruginosa* PAC 1. *J. gen. Microbiol.*, **98**, 387.

MANDRELL, R. E. AND ZOLLINGER, W. D. 1977. Lipopolysaccharide serotyping of *Neisseria meningitidis* by hemagglutination inhibition. *Infect. Immun.*, **16**, 471.

MILES, A. A., MISRA, S. S. AND IRWIN, J. O. 1938. The estimation of the bactericidal power of the blood. *J. Hyg., Camb.*, **38**, 732.

PENNER, J. L. AND HENNESSY, J. N. 1980. Passive hemagglutination technique for serotyping *Campylobacter fetus* ssp. *jejunii* on the basis of soluble heat-stable antigens. *J. clin. Microbiol.*, **12**, 732.

ROBINSON, D. A., EDGAR, W. J., GIBSON, G. L., MATCHETT, A. A. AND ROBERTSON, L. 1979. Campylobacter enteritis associated with consumption of unpasteurised milk. *Br. med. J.*, **1**, 1171.

SADOFF, J. C., ZOLLINGER, W. D. AND SIDBERRY, H. 1978. Cell surface structures of *Neisseria gonorrhoeae*. *In* Immunobiology of *Neisseria gonorrhoeae*, edited by G. F. Brooks, E. C. Gotschlich, K. K. Holmes, W. D. Sawyer and F. E. Young. American Society for Microbiology, Washington DC, p. 93.

SIDBERRY, H. D. AND SADOFF, J. C. 1977. Pyocin sensitivity of *Neisseria gonorrhoeae* and its feasibility as an epidemiological tool. *Infect. Immun.*, **15**, 628.

SKIRROW, M. B. 1977. Campylobacter enteritis: a "new" disease. *Br. med. J.*, **2**, 9.

SKIRROW, M. B. AND BENJAMIN, J. 1980. '1001' Campylobacters: cultural characteristics of intestinal campylobacters from man and animals. *J. Hyg., Camb.*, **85**, 427.

TELFER BRUNTON, W. A. AND HEGGIE, D. 1977. Campylobacter associated diarrhoea in Edinburgh. *Br. med. J.*, **2**, 956.

TREHAN, W. AND VOGT, R. L. 1978. Waterborne campylobacter gastro-enteritis—Vermont. Morbidity and Mortality, Weekly Report, Center for Disease Control, Atlanta, **27**, 207.

VINZENT, R., DUMAS, J. AND PICARD, N. 1947. Septicémie grave au cours de la grossesse, due à un vibrion. Avortement consécutif. *Bull. Acad. natn. Méd.*, **131**, 90.

WATSON, K.C., KERR, E. J. C. AND MCFADZEAN, S. M. 1979. Serology of human campylobacter infections. *J. Infect.*, **1**, 151.

ZOLLINGER, W. D. AND MANDRELL, R. E. 1977. Outer-membrane protein and lipopolysaccharide serotyping of *Neisseria meningitidis* by inhibition of a solid-phase radioimmunoassay. *Infect. Immun.*, **18**, 424.

Lab. Immunol. (1982), 9, 169–172

PHAGOCYTE RECOGNITION OF NEISSERIA GONORRHOEAE

D. F. KINANE, C. C. BLACKWELL, D. M. WEIR and F. P. WINSTANLEY
Department of Bacteriology, University Medical School, Teviot Place, Edinburgh EH8 9AG, Scotland, UK
(Received 15 February 1982)

SUMMARY Human monocytes show an increased ability to bind pilate rather than non-pilate gonococci. Polymorphonuclear leucocytes (PMN) do not discriminate between pilate and non-pilate variants. PMN, however, bind appreciably more gonococci (either pilate or non-pilate) than monocytes. The results help to explain the role of pili on gonococci as virulence factors and in the immune response.

ODUCTION

CHMENT of *Neisseria gonorrhoeae* to mucosal cells heir subsequent invasion are the initial steps in stablishment of gonococcal infection. When the isms have penetrated the mucosal barriers, the me then largely depends on their interaction with cytic cells. Attachment and uptake by polymorpho- r leucocytes (PMN) may lead to destruction of the ism. Attachment to macrophages is likely to lead to ion of an immune response by presentation of bial antigen to the lymphoid cells. The interaction en phagocytes and bacteria is clearly an important inant in the pathogenesis of the disease. Binding phagocyte membrane is dependent on the presence opriate adhesin receptor interactions between ia and phagocytes (1).

virulence of *N. gonorrhoeae* has been linked with ular colonial types, pilate types T_1 and T_2, but not non-pilate types T_3 and T_4 (2). Pilate gonococci been found to adhere more efficiently to certain tissues than non-pilate organisms, e.g. epithelial), sperm cells (4), buccal cells (5), erythrocytes (3) sue culture cells (6, 7). No difference in binding to has been observed with pilate and non-pilate occi (7, 8); attachment of gonococci to PMN s to be mediated by another surface component, kocyte association factor (9).

iew of the different roles PMN and macrophages host defence, we sought differences in the ability se cells to bind pilate and non-pilate gonococci y isolated from an infected patient.

ndence: Dr. D. M. Weir, Reader, Immunology Laboratory, ent of Bacteriology, University Medical School, Teviot Place, gh EH8 9AG, Scotland, UK.

MATERIALS AND METHODS

Bacterial Strain

Neisseria gonorrhoeae strain E757, recently isolated from a patient attending the Department of Genitourinary Medicine was used throughout these experiments. Opaque colony types 2 (T_2) and 3 (T_3) were differentiated with a Zeiss stereoscopic microscope with a double system of substage lighting and selectively subcultured and maintained on Difco GC base supplemented as described by Young (10) (GC agar). T_2 and T_3 growths, with more than 95% of the colonies stable, were employed. Bacteria were harvested from GC agar plates at 14–16 hr, suspended in Dulbecco's phosphate buffered saline (D.PBS) by gentle pipetting, washed and finally re-suspended gently in D.PBS. The pili were sheared by vortexing a suspension of gonococci in D.PBS with a Rotamixer Deluxe (Hook and Tucker Ltd., England) at maximum setting for 5 min.

Grids negatively stained with phosphotungstic acid were examined by electron microscopy (Hitachi HU12A). This confirmed the presence of pili on type 2 gonococci and their absence on type 3 and vortexed type 2 and 3 gonococci (3, 11).

Bacterial concentrations were determined at E650 nm on a CE292 spectrophotometer (Cecil Instruments, Cambridge, England) by reference to standards enumerated by microscope counts with a counting chamber. Dose response experiments indicated that the optimal concentration of gonococci for these assays was 10^8 organisms/ml and this concentration was used throughout.

Phagocytes

Blood from healthy donors with no history of gonorrhoea was collected in plastic tubes containing 10 units of heparin ml^{-1}. Monocytes and PMN were separated on Ficoll-Hypaque cushions (Ficoll, Pharmacia, London)—specific gravity 1·078—according to the method of Böyum (12). After the interface cells (monocytes and lymphocytes) were collected, the pellet containing granulocytes and red blood cells was re-suspended in 0·82% ammonium chloride to lyse the erythrocytes (13). The separated cells were finally washed twice with D.PBS.

Preparation of Monolayers

PMN and monocytes were re-suspended in Eagle's minimum essential medium (MEM) without serum, buffered at pH 7·3 with HEPES (final concentration, 30 mM; Wellcome Research Laboratories, Beckenham), and supplemented with penicillin/streptomycin (final concentration, 100 $u.ml^{-1}$ of each) and glutamine (final concentration, 2 mM) to give

82 1200-0169 $03.50

a total cell count of 2×10^5 ml. Aliquots (1 ml) of the above cell preparations were layered onto 13 mm diameter (No. 1) glass coverslips in tissue culture plates 16 mm well diameter; (Costar, 295 Broadway, Cambridge, Mass.) and incubated for 1 hr at 37°C to allow adhesion. Non-adherent cells were removed by repeated washing with D.PBS.

Binding Assay

Coverslips were overlaid with 1 ml of gonococci in D.PBS containing Ca^{++} and Mg^{++} ions (0·9 mM and 0·5 mM, respectively) and incubated for: (1) 30 min at 37°C, and (2) 2 hr at 4°C. Non-attached organisms were removed by repeated washing with D.PBS. Coverslips were air dried, fixed in methanol and stained with May Grunwald/Giemsa. Bacterial binding was estimated by counting leucocytes with bacteria attached at two or more discrete points. Duplicate coverslips were used; for each coverslip, 200 leucocytes were counted and the results expressed as the percentage of cells binding organisms.

Analysis of Results

Statistical analysis of the results was by paired sample *t* tests.

Results

Attachment of Pilate and Non-pilate Variants

Figure 1 summarises the results of three experiments comparing the attachment of pilate (T_2) and non-pilate (T_3) gonococci to PMN and macrophages. There was no significant difference in association of the two types to PMN at either 4°C or 37°C ($P > 0·5$). The association of T_2 cells with monocytes was significantly greater than that observed with T_3 cells at both 4°C ($P < 0·02$) and 37°C ($P < 0·01$). A significantly greater number of PMN than monocytes bound both T_2 and T_3 gonococci ($P < 0·01$) (table 1).

Effect of Removal of Pili

Figure 2 shows that the difference in binding of T_2 and T_3 cells to monocytes illustrated in Figure 1 was abolished by removal of pili from T_2 cells by mechanical shearing. While there was a slight overall reduction in binding of gonococci to the phagocytes following shearing treatment (table 2), there was still a significantly greater number of PMN than monocytes binding gonococci ($P < 0·05$).

Comparison of 4°C and 37°C Assays

The results for the assays done at 4°C represent attachment only as the monocytes and PMN do not phagocytose at this temperature. The 37°C assays are for attachment and phagocytosis, i.e. association. Differentiation between phagocytosis and attachment was not d but the general trends in phagocyte/gonococcal ass tion at 4°C and 37°C were consistent and suggest tha type of attachment seen at 4°C also occurs at ph logical temperatures at which phagocytosis also occu

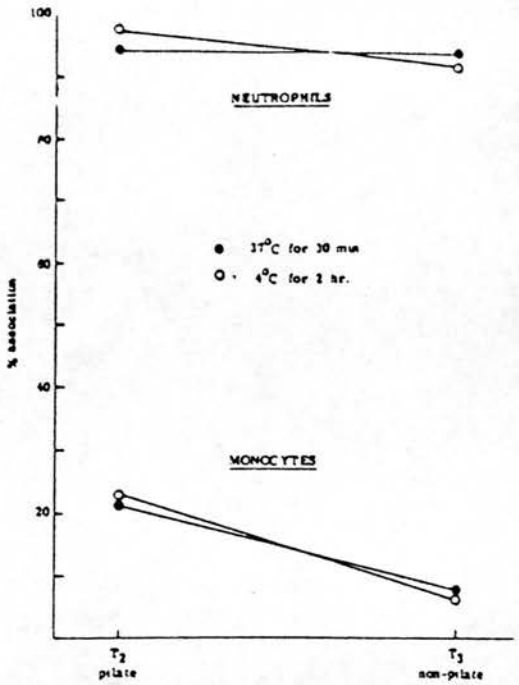


FIG. 1. Association of pilate (T_2) and non-pilate (T_3) gonococ PMN and monocytes at 37°C and 4°C. Each point represents the of three experiments.

Discussion

These results indicate that the recognition of *gonorrhoeae* by PMN and monocytes differs. Our findi agree with the observation of Swanson (7) that PMN not appear to discriminate in their ability to bind pil and non-pilate variants of the strain used. Monocytes contrast, showed an increased binding of pilate organis similar to that observed for epithelial cells (3), sperm buccal cells (5), erythrocytes (3) and tissue culture c (6, 7).

Two roles have been suggested for pili in the establi ment of gonococcal infection: (1) pili assist the organi in attaching to the mucosa (6,. 14); and (2) pili incre

Table 1 Association of pilate (T_2) and non-pilate (T_3) gonococci with PMN and monocytes at 37°C and 4°C

N. gonorrhoeae E757	*Temperature*	*Percentage binding to*			
		PMN	*Monocytes*	*% difference*	*P value*
T_2	37°C	94·17	21·17	73	<0·01
	4°C	97·33	23·67	64	<0·001
T_3	37°C	93·17	7·00	86	<0·01
	4°C	92·17	6.50	86	<0·01

Table 2 Effect of loss of pili on association of gonococci with PMN and monocytes at 37°C and 4°C

N. gonorrhoeae E757	*Temperature*	*Percentage binding to* PMN	Monocytes	% difference	*P value*
T_2 (vortexed)	37°C	75·67	10·25	65	<0·02
	4°C	61·67	6·33	55	<0·05
T_3 (vortexed)	37°C	70·25	8·33	62	<0·05
	4°C	58·58	9·33	49	<0·05

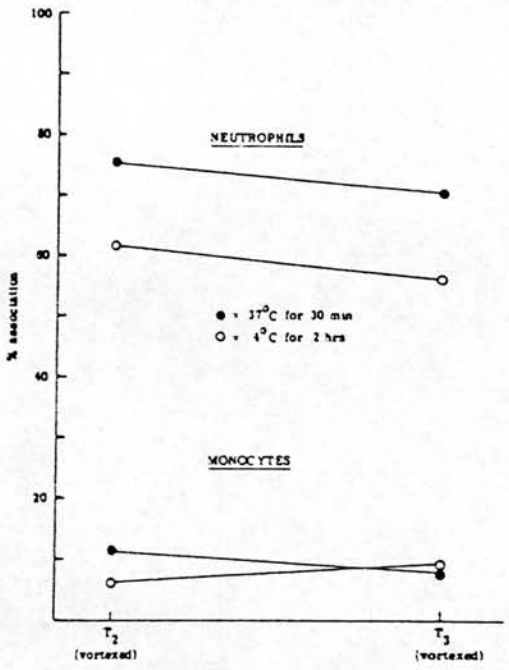


FIG. 2. The effect on binding of both T_2 and T_3 gonococci to PMN and monocytes following mechanical shearing treatment of the gonococci. Each point represents the mean of three experiments.

gonococcal resistance to phagocytosis by PMN (3, 15, 16, 17). Our findings suggest a third role in conjunction with the hypothesis of Novotny and his colleagues (1977) that "gonorrhoea appears to be a specific disease of human macrophages".

Novotny and his colleagues observed "infectious units" of gonococci in electron micrographs of pus from more than 70 infected individuals. These are clusters of organisms that appear to be undamaged and in an exponential growth state. Surrounding the gonococci is a coat made up of granules and remnants of mitochondria that are thought to be derived from macrophages. There is evidence, from labelled antibody studies, to suggest that the dense accumulation of granules protects the gonococci from humoral defences. These granular coats also seem to allow the gonococci to go unrecognised by phagocytic cells as long as the coat remains dense.

According to Novotny's hypothesis, a host is infected with both free non-multiplying gonococci and multiplying gonococci in the protective infectious units. The multiplying organisms infiltrate the mucosa and submucosa of the genitourinary tract. Free gonococci phagocytosed by PMN are destroyed, but those ingested by monocytes are believed to interfere with regulation of the phagocytic cell and multiply within it. This results in the destruction of the monocyte, the remnants of which surround the gonococci to form a new infectious unit. The results of the present study encourage us to postulate that the presence of pilar antigen on the gonococcal surface is a virulence factor that increases the probability of the bacteria becoming attached to and subsequently phagocytosed by monocytes.

The large difference we have shown in binding of gonococci (whether pilate, non-pilate or vortexed) between PMN and monocytes is most striking and contrasts with work done on *Staph. albus* in our department; in the staphylococcal studies, binding to PMN and monocytes showed no differences (19). Our results with gonococci may also provide an explanation for gonorrhoeal recurrence additional to the theory of broad heterogeneity of the gonococcal antigens (20, 21, 22). An ineffective immune response to gonococcal infection may be explained by the much greater ability of PMN to take up the organism in comparison with the smaller numbers that are recognised and taken up by monocytes. If these are largely incorporated into "infectious units" and survive, the presentation of gonococcal antigen by the monocytes to the lymphocytes will be reduced in comparison with other types of antigens. Pili are not readily demonstrated on gonococci in human tissues (23, 24). If proteolytic enzymes in pus and tissues remove these from the surface of the organism, the probability of attachment to monocytes would be further reduced. As pili seem to enhance attachment to epithelial cells and macrophages, loss of pili would be likely to limit colonisation and to reduce the possibility of the development of infectious units in macrophages with a consequent reduction in virulence.

ACKNOWLEDGEMENTS

We are grateful to Mrs. Marilyn Cole for help in typing the manuscript and to Professor J. G. Collee for his advice in the preparation of the manuscript. This investigation was supported by Grant No. K/MRS/50/C22 from the Biomedical Research Committee, Scottish Home and Health Department and by Grants G81 103811 SB and G979/74/S from the Medical Research Council.

M. (1980). Surface carbohydrates and lectins in cellular n. *Immunology Today*, **1**, 45.
S. Jr., Peacock, W. L. Jr., Deacon, W. E., Brown, L. and I. (1963). *Neisseria gonorrhoeae*. I. Virulence genetically lonal variation. *J. Bacteriol.*, **85**, 1274.
. A. P. Jr. and Sawyer, W. D. (1973). Role of pili in the f *Neisseria gonorrhoeae*. *Infect. Immun.*, **8**, 255.
lmquest, A. N., Swanson, J., Buchanan, T. M., Wende, Williams, R. P. (1974). Differential attachment by pilated ilated *Neisseria gonorrhoeae* to human sperm. *Infect.* 897.
E. M. and Wilson, C. (1977). Variations in buccal cell f *Neisseria gonorrhoeae*. *Infect. Immun.*, **16**, 709.
I. (1973). Studies on gonococcus infection. IV. Pili: their achment of gonococci to tissue culture cells. *J. Exp.* 571.
., King, G. and Zeligs, B. (1975). Studies on gonococcus VIII. ^{125}Iodine labelling of gonococci and studies on *ro* interactions with eukaryotic cells. *Infect. Immun.*, **11**,
., Sparks, E., Zeligs, B., Siam, M. and Parrott, C. (1974). n gonococcus infection. V. Observations on *in vitro* s of gonococci and human neutrophils. *Infect. Immun.*,
., Sparks, E., Young, O. and King, G. (1975). Studies on s infection. X. Pili and leukocyte association factor as in interactions between gonococci and eukaryotic cells *in* t. *Immun.*, **11**, 1352.
, Patterson, I. C. and McDonald, D. R. (1976). Rapid ate utilisation test for the identification of *Neisseria* e. *Brit. J. Vener. Dis.*, **52**, 172.
R. S., Fulbright, R. S., Eads, M. E. and Sawyer, W. D. hylenediaminetetracetic acid-sensitive antiphagocytic Neisseria gonorrhoeae. *Infect. Immun.*, **15**, 817.
. (1968). Isolation of leucocytes from human blood. bservation. Methyl cellulose dextran and Ficoll as erythrocyte aggregating agents. *J. Clin. Lab. Invest.*, **21**, Suppl. 97, 31.
13. Boyle, W. (1968). An extension of the ^{51}Cr release assay for the estimation of mouse cytotoxins. *Transplantation*, **67**, 761.
14. Ward, M. E., Watt, P. J. and Robertson, J. N. (1974). The human fallopian tube: a laboratory model for gonococcal infection. *J. Infect. Dis.*, **129**, 650.
15. Swanson, J., Kraus, S. J. and Gotschlich, E. C. (1971). Studies on gonococcus infection. I. Pili and zones of adhesion: their relation to gonococcal growth patterns. *J. Exp. Med.*, **134**, 886.
16. Thongthai, C. and Sawyer, W. D. (1973). Studies on the virulence of *Neisseria gonorrhoeae*. 1. Relation of colonial morphology and resistance to phagocytosis by polymorphonuclear leukocytes. *Infect. Immun.*, **7**, 373.
17. Ofek, I., Beachey, E. H. and Bisno, A. L. (1974). Resistance of *Neisseria gonorrhoeae* to phagocytosis: relationship to colonial morphology and surface pili. *J. Infect. Dis.*, **129**, 310.
18. Novotny, P., Short, J. A., Hughes, M., Miler, J. J., Syrett, C., Turner, W. H., Harris, J. R. W. and Maclennan, I. P. B. (1977). Studies on the mechanisms of pathogenicity of *Neisseria gonorrhoeae*. *J. Med. Microbiol.*, **10**, 347.
19. Glass, E. J., Stewart, J. and Weir, D. M. (1981). Presence of bacterial binding "lectin-like" receptors on phagocytes. *Immunology*, **44**, 529.
20. Kasper, D. L., Rice, P. A. and McCormack, W. M. (1977). Bactericidal antibody in genital infection due to *Neisseria gonorrhoeae*. *J. Infect. Dis.*, **135**, 243.
21. McMillan, A., McNeillage, G., Young, H. and Bain, S. S. R. (1979). Secretory antibody response of the cervix to infection with *Neisseria gonorrhoeae*. *Br. J. Vener. Dis.*, **55**, 265.
22. McMillan, A., McNeillage, G., Young, H. and Bain, S. S. R. (1979). Serum immunoglobulin response in uncomplicated gonorrhoea. *Br. J. Vener. Dis.*, **55**, 5.
23. Novotny, P., Short, J. A. and Walker, P. D. (1975). An electron-microscope study of naturally occurring and cultured cells of *Neisseria gonorrhoeae*. *J. Med. Microbiol.*, **8**, 413.
24. Evans, B. A. (1977). Ultrastructural study of cervical gonorrhoea. *J. Infect. Dis.*, **136**, 248.

Teviot Scientific Publications Ltd
31 Montpelier Park
Edinburgh EH10 4LX, UK

Reprinted from the BRITISH MEDICAL JOURNAL
3 July 1982, 285, 7 - 9

ABO blood group, secretor state, and susceptibility to recurrent urinary tract infection in women

D F KINANE, C CAROLINE BLACKWELL, R P BRETTLE, D M WEIR, F P WINSTANLEY, R A ELTON

Abstract

ABO blood group and secretor state was determined in 319 women with recurrent urinary tract infection and compared with those of a control group of 334 women of similar age ranges. Women of blood groups B and AB who are non-secretors of blood group substances showed a significant relative risk of recurrent urinary tract infection of 3·12 (95% confidence limits, 1·49 and 6·52) in comparison with other types. This appears to be a genuine example of synergy in which absence of anti-B isohaemagglutinin and secretor substances combines to give an increased risk of recurrent urinary tract infection.

Determination of blood group and secretor state may provide additional information in identifying those at risk.

Department of Bacteriology, University of Edinburgh Medical School, Edinburgh EH8 9AG

D F KINANE, BDS, research fellow
C CAROLINE BLACKWELL, PHD, lecturer
D M WEIR, MD, FRCP, reader
F P WINSTANLEY, BSC, research associate

Department of Infectious Diseases, City Hospital, Edinburgh EH10 5SB

R P BRETTLE, MRCP, senior registrar

Medical Computing and Statistics Unit, University of Edinburgh Medical School, Edinburgh EH8 9AG

R A ELTON, PHD, lecturer

251/82

Introduction

Correlation between ABO blood group and susceptibility to certain infectious diseases is well documented.[1-6] In studies of a Chilean population, patients of blood group B had 50% greater probability than those of other blood groups of contracting *Escherichia coli* urinary tract infections.[4]

The antigens of ABO blood groups are present in two forms: (*a*) alcohol-soluble in tissues of all subjects, and (*b*) water-soluble in most body fluids and organs of secretors. The non-secretor state has been linked with the susceptibility to certain infectious agents.[7-9] Haverkorn and Goslings[7] found that patients in blood groups other than O and non-secretor patients were most susceptible to rheumatic fever and rheumatic heart disease, and carriage of group A streptococci was observed more often in non-secretors.

We analysed the distribution of ABO blood groups and secretor state among women with recurrent urinary tract infections in Edinburgh.

Subjects

Regular attenders at the pyelonephritis clinic of the City Hospital, Edinburgh, were asked to provide a specimen of blood and saliva for determination of blood group and secretor state. All patients in this survey were girls and women aged 10-80 (mean 49) years. None had predisposing factors such as stones.

The patients were divided prospectively into two categories:

Patients with pyelonephritis—Those with unequivocal radiological evidence of chronic pyelonephritis—that is, focal loss of parenchyma associated with distortion of calyces—or those with a history and clinical and laboratory findings consistent with acute pyelonephritis.

Patients with uncomplicated urinary tract infections—Those patients with normal appearance on intravenous pyelograms, but with appreciable bacteriuria (10^5 organisms/ml) and a history of recurrent urinary tract infections.

Controls—Control data were produced by testing 334 samples of plasma from female donors (age range 18-60) from the survey area for the presence of secretor substances. Further control data for distribution of blood groups were available for 6662 donors from the same area as that from which our patients were drawn.[10]

Methods

Blood from each patient was collected at the clinic and stored in heparinised tubes. The saliva from each patient was boiled for 20 min, centrifuged at $5000 \times g$ for 10 min, and the supernate stored briefly at 4°C until tested for the presence of blood group antigen. Blood group was determined by agglutination tests in plastic wells. Secretor state was determined as described by Mollinson[11] with saliva or

plasma samples. Saliva or plasma from secretors and non-secretors representative of the four blood groups were used as controls for each experiment. Agglutinins used were anti-A and anti-B sera and *Ulex europaeus* lectin. The red blood cells used were A_2, B, and O.

Linear logistic multiple regression[12] was used to compare the ABO blood group and secretor state categories of the study and control groups. This also gave estimates and confidence limits for relative risk of urinary tract infection between categories. χ^2 tests were used for analysis of two-way frequency tables.

Results

No significant differences in the proportion of secretors or the distribution of ABO blood groups were found between the two diagnostic categories of patients with urinary tract infections; all 319 patients were considered as one group for comparisons with controls. The table shows the distribution of different blood groups and secretor state for the study group and for the smaller series of controls in whom secretor status had been determined. For the study group, the table shows also the ratios of observed prevalences to those expected from the pattern found in controls.

Distribution of blood groups in study group and in two groups of controls, those in whom secretor state was determined (group 1) and those in whom it was unknown (group 2) (numbers in parentheses are ratios of distribution found in study group to that expected from control data)

	Blood group			
	O	A	B	AB
Control group 1 (n = 334):				
Secretors	124	74	35	12
Non-secretors	49	30	7	3
Study group (n = 319):				
Secretors	110 (0·93)	63 (0·88)	32 (0·96)	7 (0·64)
Non-secretors	52 (1·11)	28 (0·98)	18 (2·69)	10 (3·49)
Control group 2 (n = 6662)	3323	2410	715	214

To test the dependence of risk of urinary tract infection on ABO blood group or secretor state, a linear logistic multiple regression was carried out on the two samples. This examines the influence of the three main effects (anti-A, anti-B, and secretor state) and their interactions on the proportion of subjects with urinary tract infection, using approximate χ^2 tests. Presence or absence of anti-A was found not to have a significant effect, but there was a significant interaction between the anti-B and secretor effects; subjects lacking both anti-B and secretor substance—that is, those of blood groups B and AB who are non-secretors—had a higher risk of recurrent urinary tract infection, whereas subjects lacking only one of these two did not differ signifi-

cantly in risk from those with both factors present. Thus the data fit extremely well ($\chi^2 = 1{\cdot}86$, 6 df) to a model in which the non-secretor in blood groups B and AB have a highly significant excess risk ($\chi^2 = 9{\cdot}12$, 1 df, $p < 0{\cdot}01$) over all other types. The relative risk of urinary tract infection for these subjects as compared with other types was estimated as 3·12, with 95% confidence limits of 1·49 and 6·52. This appears to be a genuine example of synergy with absence of both anti-B and secretor substance being necessary to give an increased risk of urinary tract infection. The ratios of observed to expected prevalence in the table show this finding clearly, with only those for non-secretors in blood groups B and AB differing substantially from unity.

The larger series of controls (in whom only ABO group and not secretor state was known) did not differ significantly from the smaller series in their blood group frequencies, but showed a highly significant difference ($\chi^2 = 16{\cdot}34$, 3 df, $p < 0{\cdot}001$) from the patients with urinary tract infections (table) (14% in blood groups B and AB *v* 21%). This confirms previous findings and is consistent with the suggested model in which non-secretors in blood groups B and AB have an increased likelihood of recurrent urinary tract infection. The proportion of anti-B negative patients in the study group is inflated by the excess prevalence of those who are also non-secretors, even though anti-B negative secretors are not at a higher risk. Thus, absence of anti-B when considered alone shows a significant risk relative to presence of anti-B (95% confidence limits of 1·24 and 21·7 (table)). This risk is lower than the threefold increase in risk found for non-secretors in blood groups B and AB, because the anti-B negative group is "diluted" with anti-B negative secretors.

Discussion

The highly significant excess of non-secretors in blood groups B or AB in the study group strongly suggests a synergistic link between the absence of anti-B isohaemagglutinin and secretor substances and an increased risk of recurrent urinary tract infection in women. In the controls the prevalence of non-secretors of 26·6% reflects an increased incidence of non-secretors found in Scottish and Irish ethnic groups compared with that reported for other regions in the United Kingdom.[13] This highlights the need to use local control groups in surveys of this nature.

The association of infections with ABO blood group and secretor state[1-9] has led to investigation of the mechanisms concerned.[6 14 15] Many bacteria cross-react with ABO blood groups.[14-20] Drach *et al*[18] found A and B blood group activity in 47% of 34 urinary tract pathogens. Isohaemagglutinins may have an important protective role against these cross-reacting bacteria.[15 19] Our finding that individuals of blood groups B and

AB with no anti-B isohaemagglutinins are more susceptible to urinary tract infection is interesting in that Drach[19 20] found antigens like those of blood groups A and B on urinary tract pathogens, often with both group A and group B activity on the same bacterium. These workers noted that anti-B titres were sometimes raised in the disease but anti-A ones were not, suggesting that the antigen to blood group A was less immunogenic on the bacteria or that there were deficiencies in recognition of antigens of blood group A. Springer *et al*[16] found *E coli* 086 B:7 to be highly interactive with the blood group: thus growth was retarded in the presence of anti-B sera; if complement was added a bactericidal reaction ensued. Isohaemagglutinins may also interact with blood group like antigens on bacterial cell walls to inhibit attachment to uroepithelial or periurethral cells. Thus persons capable of producing anti-B isohaemagglutinins may have a greater degree of protection against urinary tract infection. In general, the extent of bacterial cross-reaction with ABO blood groups suggests that a selective advantage linked to ABO blood group might operate in combating infections.

Secretor state is associated with certain differences in immunoglobulin concentrations in that anti-B serum IgA and (in white people) serum IgG concentrations are lower in non-secretors than in secretors.[21 22] This suggests that non-secretors may have less effective immune protection than secretors and is consistent with our findings that non-secretors were more susceptible to recurrent urinary tract infection. This probably entails the participation of other factors, and the finding that excretion of blood group substances in urine is an active process in the kidney and not a simple filtration process[23] suggests that these substances may have a protective part to play in secretions. Boat *et al*[24] found that ABH blood group substances of saliva inhibited haemagglutination of influenza B virus and suggested that some of these substances may interfere with access of influenza virus to binding sites. One of the protective roles of these blood group substances may be their ability to occupy or in some way interfere with binding sites either on the bacterium or on the epithelial cell with possible effects on bacterial colonisation and subsequent invasion and infection; this appeared to be the case in our studies on diabetic mice.[25]

Determination of blood groups and secretor state may provide additional information in identifying individuals who are at risk. In children under the age of 5 the combination of infection and vesicoureteral reflux predisposes to renal scarring.[26] Few if any tests are available for providing prognostic information for adults with a normal urinary tract and a history of recurrent infection. Blood group and secretor state may be of relatively minor importance in relation to all other aspects of susceptibility.

Nevertheless, it may be worthwhile for the clinician to take account of ABO and secretor state in addition to already established factors in the assessment of patients with urinary tract infection when considering their long-term susceptibility and management. Meanwhile, there is a good case for research workers concerned with mechanisms of microbial pathogenicity to add this consideration to an extending list of host factors that bear on host-parasite interactions.

This work was supported by grant K/MRS/50/C22 from the Biomedical Research Committee, Scottish Home and Health Department and by grants G81/103811/SB and G979/74/S from the Medical Research Council.

We thank the Blood Transfusion Service, Royal Infirmary, Edinburgh for supplying reagents for blood grouping and determination of secretor state and for undertaking random verification checks on selected saliva (50) and plasma (48) samples; Dr J McMurdoch and Dr J A Gray for permission to study their patients; the doctors and staff of Ward 14, City Hospital, Edinburgh; and Professor J G Collee and Messrs A McGowan and G Neill for their help and advice.

References

[1] Barua D, Paguio AS. ABO blood group and cholera. *Ann Hum Biol* 1977; **4**:489-92.

[2] Socha W, Bilinska M, Kaczera Z, Padjak E, Stankiewicz D. *Escherichia coli* and ABO blood groups. *Folia Biol (Krakow)* 1969;**17**:259-69.

[3] Robinson MG, Tolchrin D, Halpern C. Enteric bacterial agents and the ABO blood groups. *Am J Hum Genet* 1971;**23**:135-45.

[4] Cruz-Coke R, Parades L, Montengro A. Blood groups and urinary microorganisms. *J Med Genet* 1965;**2**:185-8.

[5] Foster MT, Labrum AH. Relation of infection with *Neisseria gonorrhoeae* to blood groups. *J Infect Dis* 1976;**133**:329-30.

[6] Miler JJ, Novotny P, Walker PD, Harris JRW, MacLennan IPB. *Neisseria gonorrhoeae* and ABO isohaemagglutinins. *Infect Immun* 1977; **15**:713-9.

[7] Haverkorn MJ, Goslings WRO. Streptococci ABO blood groups and secretor status. *Am J Hum Genet* 1969;**21**:360-75.

[8] Pradhan AC, Chawla TN, Samuel KC, Pradhan S. The relationship between periodontal disease and blood groups and secretor status. *J Periodont Res* 1971;**6**:194-300.

[9] Chaudhuri A, Das Adchickary CR. Possible role of blood group secretory substances in the aetiology of cholera. *Trans R Soc Trop Med Hyg* 1978;**72**:664-5.

[10] Kopeć AC. *The distribution of the blood groups in the United Kingdom.* Oxford: Oxford University Press, 1970:26.

[11] Mollinson PL. *Blood transfusion in clinical medicine.* 6th ed. Oxford: Blackwell, 1979:414-82.

[12] Armitage P. *Statistical methods in medical research.* Oxford: Blackwell, 1971:375.

[13] Lincoln PJ, Dodd BE. Variation in secretor and Lewis type frequencies within the British Isles. *J Med Genet* 1972;**9**:43-5.

[14] Springer GF, Williamson P, Brandes WC. Blood group activity of Gram-negative bacteria. *J Exp Med* 1961;**113**:1177-93.

[15] Muschel L, Osawa E. Human blood group substances B and *Escherichia coli* 086. *Proc Soc Exp Biol Med* 1959;**101**:614-7.
[16] Springer GF. Importance of blood group substances in interactions between man and microbes. *Ann NY Acad Sci* 1970;**169**:134-52.
[17] Springer GF. Blood group and Forssman antigenic determinants shared between microbes and mammalian cells. *Prog Allergy* 1971;**15**:9-77.
[18] Drach GW, Reed WP, Williams RC. Antigens common to human and bacterial cells: urinary tract pathogens. *J Lab Clin Med* 1971;**78**:725-35.
[19] Check JH, O'Neill EA, O'Neill KE, Fuscaldo KE. Effect of anti-B antiserum on the phagocytosis of *Escherichia coli*. *Infect Immun* 1972;**6**: 95-6.
[20] Drach GW. Antigens common to human and bacterial cells: tolerance or resistance to urinary and surgical infections. *Surg Forum* 1971;**22**:67-8.
[21] Grundbacher FJ, Shreffler DC. Effects of secretor, blood and serum groups on isoantibody and immunoglobulin levels. *Am J Hum Genet* 1970;**22**:194-202.
[22] Grundbacher FJ. Immunoglobulins, secretor status and the incidence of rheumatic fever and rheumatic heart diseases. *Hum Hered* 1972;**25**: 399-404.
[23] Kalinowski M. Urinary elimination of group substances ABO(H) in different renal diseases. *Folia Haematol* (*Liepz*) 1972;**100**:144-63.
[24] Boat TF, Davis J, Stern RC, Cheng PW. Effect of blood group determinants on binding of human salivary mucous glycoproteins to influenza virus. *Biochim Biophys Acta* 1978;**540**:127-33.
[25] Weir DM, Blackwell CC, McLean CA. Impaired bacterial binding to peritoneal exudate cells from mice with alloxan induced diabetes. *Journal of Clinical and Laboratory Immunology* 1981;**5**:37-40.
[26] Rolleston GL, Shannon FT, Utley JF. Relationship of infantile cyto-ureteric reflux to renal damage. *Br Med J* 1970;i:460-3.

(*Accepted 19 April 1982*)

Printed in Great Britain by Bourne Offset (Iver) Ltd., Iver, Bucks.

Br J Vener Dis 1983;59:44-6

Blood group, secretor status, and susceptibility to infection by Neisseria gonorrhoeae

D F KINANE, C C BLACKWELL, F P WINSTANLEY, AND D M WEIR
From the Department of Bacteriology, University of Edinburgh Medical School, Edinburgh

SUMMARY To determine whether the presence or absence of anti-B isohaemagglutinin in individuals of blood group B increases their susceptibility to gonococcal infections 567 new patients attending a sexually transmitted disease clinic were screened for blood group and secretor status. Of the patients with blood group B, 20·1% had gonorrhoea and 12% had not. A higher percentage (20·9%) of patients with no anti-B isohaemagglutinin had gonorrhoea compared with those without (12·1%). There was, however, no synergy between the absence of anti-B isohaemagglutin and non-secretion of water-soluble blood group B antigen. Further research is needed to determine the underlying host-parasite interactions responsible for the increased susceptibility to gonorrhoea in these individuals.

Introduction

Over the past six years there have been reports of increased susceptibility of individuals of blood group B to infection with *Neisseria gonorrhoeae*.[1,2] Foster and Labrum[1] suggested that the presence or absence of anti-B isohaemagglutinin may be the causal factor for the reported increase in susceptibility to gonorrhoea of individuals with blood group B. In a recent study we found that individuals of blood groups B and AB — that is, those with no anti-B isohaemagglutinin — who were also non-secretors of blood group antigen were significantly more susceptible to urinary tract infection.[3] To determine whether there is a synergistic effect between those two host factors and susceptibility to gonococcal infection we screened patients attending a department of genitourinary medicine for blood group and secretor status, and we related the data to the occurrence of gonorrhoea in these patients.

Patients and methods

During a three-month period all new patients attending the department of genitourinary medicine at the Royal Infirmary, Edinburgh, were asked to provide a specimen of blood and saliva for determination of blood group and secretor status.

Address for reprints: Dr D F Kinane, Department of Bacteriology, University of Edinburgh Medical School, Teviot Place, Edinburgh EH8 9AG

Accepted for publication 29 August 1982

Control data for blood group frequency were available for 6662 donors from the same geographical area.[4] Secretor status control data were produced by testing 334 plasma specimens from blood donors in the survey area.

SCREENING PROCEDURES

Blood from each patient was collected at the clinic and stored in heparinised tubes. The saliva from each patient was boiled for 20 minutes, centrifuged at 500 × *g* for 10 minutes and the supernate stored briefly at 4°C until tested for the presence of blood group antigen.

Blood group was determined by agglutination tests in plastic wells (WHO plates). Secretor status was determined as described by Mollinson[5] with saliva or plasma samples. Samples of saliva or plasma from secretors and non-secretors representative of the four blood groups were used as controls for each experiment. Agglutinins used were anti-A and anti-B sera and *Ulex europaeus* lectin. The red blood cells used were of groups A_1, B, and O. The Blood Transfusion Service, Royal Infirmary, Edinburgh, kindly supplied the reagents for blood grouping and determination of secretor status and also performed random verification tests on 50 selected saliva samples and 46 plasma samples.

Results

The distribution of blood groups of patients with and without gonorrhoea and of the controls is given in

TABLE I *Distribution of ABO blood groups in 567 patients with and without gonorrhoea and controls*

Blood group	Culture results for N gonorrhoeae		Total No (%) of patients	No (%) of controls[4]
	No (%) negative	No (%) positive		
O	247(50·3)	35(46·1)	282(49·7)	3323(49·9)
A	176(35·8)	23(30·3)	199(35·1)	2410(36·2)
B	59(12·0)	16(21·1)	75(13·2)	715(10·7)
AB	9(1·8)	2(2·6)	11(1·9)	214(3·2)
Total	491(99·9)	76(100·1)	567(99·9)	6662(100·0)

table I. The blood group frequencies of the controls and the total number of patients attending the clinic showed no significant differences ($\chi^2 = 5 \cdot 864$, $p > 0 \cdot 1$). The frequency of blood group B in these patients with gonorrhoea was 21·1% compared with 12% in those without. This difference was not, however, significant ($\chi^2 = 5 \cdot 103$, $p > 0 \cdot 1$). When the frequency of blood group B in the patients with gonorrhoea was compared (21·1%) with that of the controls (10·7%) the difference ($\chi^2 = 8 \cdot 404$, $p < 0 \cdot 05$) was significant.

The effect of the presence or absence of anti-B on susceptibility to gonorrhoea is shown in table II. A higher percentage (20·9%) of patients with no anti-B isohaemagglutinin had gonorrhoea compared with those with anti-B isohaemagglutinin (12·1%) ($\chi^2 = 4 \cdot 947$, $p < 0 \cdot 05$). Using the relative risk method of Woolf[6] we found that the relative risk of gonorrhoea was 1·93 for individuals without anti-B isohaemagglutinin. This means that they are 93% more susceptible to gonorrhoea than persons with anti-B isohaemagglutinin.

The distribution of secretor status for patients with and without gonorrhoea and controls is given in table III. No significant differences were noted between the total patients and the controls ($\chi^2 = 0 \cdot 917$, $p > 0 \cdot 1$) nor between those patients with and without gonorrhoea ($\chi^2 = 0 \cdot 168$, $p > 0 \cdot 5$).

TABLE II *Analysis of data from table I by presence or absence of anti-B isohaemagglutinin*

Anti-B	Culture results for N gonorrhoeae		Total
	No (%) negative	No (%) positive	
Present (O and A)	423(87·9)	58(12·1)	481(100)
Absent (B and AB)	68(79·1)	18(20·9)	86(100)
Total	491	76	567

Discussion

Our findings confirm those of others[1,2] that individuals of blood group B are more susceptible to gonococcal infection and further suggest that the absence of anti-B isohaemagglutinin is important.

An increase in the number of non-secretors among patients with gonococcal infections had been predicted, but this was not observed. Although there was a significant increase in the number of blood group B individuals among the infected patients, there was no synergy between the absence of anti-B isohaemagglutinin and non-secretion of water soluble blood group B antigen comparable to that found in patients with urinary tract infections.[3]

The underlying host-parasite interactions responsible for the increased susceptibility of individuals with no anti-B isohaemagglutinin to gonococcal infection are not yet known; they appear, however, to differ from those involved with other Gram-negative, urinary tract pathogens. Accordingly, we are currently investigating the role of anti-B isohaemagglutinin in normal human serum acting as an opsonin or as bactericidal antibody, gonococcal interactions with human phagocytic cells of the different blood groups, and differences in the attachment of gonococcal strains to epithelial cells from individuals with different ABO blood groups.

We are most grateful to Dr D H H Robertson and his colleagues in the department of genitourinary medicine, Royal Infirmary, Edinburgh, for their assistance in the collection of blood and saliva specimens, to Dr H Young for the results of bacteriological investigations, and to Dr R A Elton for assistance with the statistical analysis of our data. We also thank Professor J G Collee for his advice in

TABLE III *Distribution of secretor status for patients with and without gonorrhoea and controls*

Secretor status	Culture results for N gonorrhoeae		Total No (%) of patients	No (%) of controls
	No (%) negative	No (%) positive		
Secretor	344(70·1)	55(72·4)	399(70·4)	245(73·4)
Non-secretor	147(29·9)	21(27·6)	168(29·6)	89(26·6)
Total	491(100)	76(100)	567(100)	334(100)

preparation of the manuscript and Mrs M Cole for its typing. This investigation was supported by grant K/MRS/50/C22 from the Biomedical Research Committee, Scottish Home and Health Department, and by grants G81/103811/SB and G979/64/S from the Medical Research Council.

References

1. Foster MT, Labrum AH. Relation of infection with *Neisseria gonorrhoeae* to ABO blood groups. *J Infect Dis* 1976;133:329.
2. Miler JJ, Novotny P, Walker PHD, Harris JRW, MacLennan IPB. *Neisseria gonorrhoeae* and ABO isohaemagglutinins. *Infect Immun* 1977;15:713.
3. Kinane DF, Blackwell CC, Brettle RP, Weir DM, Winstanley FP, Elton RA. ABO blood groups, secretory state, and susceptibility to recurrent urinary tract infection in women. *Br Med J* 1982;285:7.
4. Kopec AC. *The Distribution of the Blood Groups in the United Kingdom*. London, New York, Toronto: Oxford University Press, 1970:26.
5. Mollinson PL. *Blood Transfusion in Clinical Medicine*. 6th ed. Oxford: Blackwell, 1979:414.
6. Woolf B. On estimating the relation between blood group and disease. *Ann Hum Genet* 1954/55;19:251.

J. Clin. Lab. Immunol. (1983), 4, 173–178

ABO BLOOD GROUP AND SUSCEPTIBILITY TO GONOCOCCAL INFECTION. I. FACTORS AFFECTING PHAGOCYTOSIS OF *NEISSERIA GONORRHOEAE*

C. C. BLACKWELL, M. KOWOLIK*, F. P. WINSTANLEY, D. F. KINANE, D. M. WEIR, J. A. LAW and Y. L. HO WANG CHOK

*Department of Bacteriology, University of Edinburgh Medical School, Teviot Place, Edinburgh, and *Department of Oral Medicine, University of Edinburgh, U.K.*

(Received 24 June 1982)

SUMMARY The effect of opsonization of *Neisseria gonorrhoeae* by isohaemagglutinins from normal serum on attachment to human polymorphonuclear leukocytes (PMN) was investigated. No significant differences between sera from blood groups O, A, B or AB were found. Differences in attachment of gonococci were related to differences in lipopolysaccharide detected by sensitivity to R-type pyocines of *Pseudomonas aeruginosa*. Non-pilate variants of each test strain markedly stimulated nitroblue tetrazolium (NBT) reduction in PMN, but their pilate variants were essentially inactive in the NBT test.

INTRODUCTION

THE establishment of an infection by *Neisseria gonorrhoeae* depends on a variety of factors including the ability of the bacteria to attach to the appropriate target cells, their uptake and destruction within phagocytic cells, and the effects of serum components on their viability and opsonization. A higher incidence of gonorrhoea among individuals of blood group B has been reported (1, 2), and a theoretical explanation is that the anti-A isohaemagglutinin characteristic of B serum may be less effective as an opsonin than the anti-B isohaemagglutinin which occurs in the sera of persons of groups A and O (1).

In this study we have investigated the effects of isohaemagglutinins in the sera of individuals of groups A, B, AB and O on the attachment to polymorphonuclear leukocytes (PMN) of gonococcal strains with different cell envelope characteristics. The effects of differences in bacterial cell envelope structure and composition as reflected by variation in complexity of pyocine receptors of the lipopolysaccharide (LPS) (3) have been examined as factors that may affect attachment of non-opsonized bacteria to the PMN membrane or the subsequent phagocytosis and killing of the bacteria.

In an earlier study with one of the strains used here we have shown that human PMN do not discriminate in their binding ability between pilate and non-pilate variants of the strain of *N. gonorrhoeae* tested (4), though these variants differ in their infectivity. Either (1) the binding mechanism studied does not reflect a more significant surface event *in vivo*, or (2) intracellular events within the PMN significantly determine the outcome of the challenge. To assess the effects of variation in the cell surface of the organism on the metabolism of the PMN with implications for the survival of the gonococci, we have tested the NBT response induced by pilate and non-pilate variants of various pyocine types.

Correspondence: Dr. C. C. Blackwell, Department of Bacteriology, University Medical School, Teviot Place, Edinburgh.

MATERIALS AND METHODS

Bacteria

The isolates of *N. gonorrhoeae* used are listed in Table 1. Strains 9 and F62 are laboratory cultures; and strains 7422 and 7502 from disseminated gonococcal infection were obtained from Dr. Joan S. Knapp, Neisseria Reference Laboratory, United States Public Health Service, Seattle, Washington. Eight others are clinical isolates from patients attending the Department of Genito-Urinary Medicine, Royal Infirmary, Edinburgh. The pyocin types of these isolates were determined by the method previously described (3). Pilate colony type T1 and non-pilate colony type T4 variants of these strains were differentiated with a Zeiss stereoscopic microscope with a double system of substage lighting and selectively subcultured and maintained on Difco GC agar base supplemented as described by Young *et al.* (5). Bacteria were harvested from these plates at 14–16 hr, suspended in Hanks' balanced salt solution (HBSS) by gentle pipetting, washed and finally resuspended in HBSS.

Sera

Serum from individuals of groups A, B, O and AB with no history of gonorrhoea was stored at −20°C in 0·5 ml aliquots and thawed immediately before use. For some experiments sera inactivated by heating at 56°C for 30 min were used.

Phagocytosis Assay

Blood was collected from a healthy group O donor with no history of gonorrhoea. Volumes of blood (7 ml) were collected in heparinized (10 iu/ml) plastic tubes to which 2 ml of 5% dextran (Sigma Chemicals) in phosphate buffered saline (PBS) pH 7·2 was added.

0141-2760/83/0400-0173 $04.50

Table 1 Pyocin types of *N. gonorrhoeae* strains

N. gonorrhoeae strain	*R1 strain*					*Unclassified strain*		*R2 strain*			*R3 strain*	*R5 strain*	
	ISD	*2285*	*IS4*	*IS6*	*IS8*	*R205*	*9579*	*ISB*	*R21*	*430*	*ISE*	*ISA*	*ISC*
Group I													
E757	−	−	−	−	−	−	+	−	−	−	−	+	±
M9131	−	−	−	−	−	−	+	−	−	−	−	+	+
7502	−	−	−	−	−	−	−	−	−	−	−	±	−
Group II													
F62	+	±	+	+	+	+	+	−	−	−	−	+	+
9	+	−	+	+	+	+	+	−	−	−	−	+	+
M8865	+	±	+	+	+	+	+	−	−	−	−	+	+
E760	+	−	+	+	+	+	+	−	−	−	−	+	+
P152	+	−	+	+	+	+	+	−	−	−	−	+	+
M6967	+	−	+	+	+	+	+	−	−	−	−	+	+
7422	+	−	+	+	+	+	+	−	−	−	−	+	+

+ inhibition.
± inhibition with some growth.
− no inhibition.

Erythrocytes were sedimented at 37°C and the leukocyte rich portion was centrifuged at 380 g for 10 min. The leukocytes were washed twice with heparinized saline and suspended in HBSS. The cell suspension was counted in a haemocytometer and the concentration adjusted to approximately 10^7 PMN/ml.

Bacterial suspensions (approximately 10^8/ml) were prepared in HBSS. The numbers of viable organisms in suitable dilutions in HBSS were determined by colony counts on GC agar.

The phagocytosis assay was essentially as described by van Furth and co-workers (6). Leukocytes (10^7/ml) and bacteria (10^8/ml) were mixed in equal volumes in plastic tubes with 0·2 ml (10%) of the appropriate serum and incubated at 37°C with rotation (16 rev/min). Aliquots of 0·4 ml were removed at 0, 10 and 30 min and added to 10 ml ice cold HBSS to prevent further phagocytosis. The leukocytes were sedimented by centrifugation at 380 g for 10 min.

Bacterial suspensions were opsonized for 30 min with serum 10% (v/v) from a healthy individual of blood group A, B or O with no history of gonorrhoea. For the control, the serum was replaced by HBSS.

For tests at each time interval, 0·5 ml samples of the pelleted leukocytes were spun onto glass slides in a cytocentrifuge (Shandon Elliot Cytospin) at 500 rpm. The slides were then studied by light microscopy. The number of leukocytes containing bacteria in vacuoles or adherent to the cell surface were expressed as a percentage of the 200 leukocytes counted. The number of bacteria per cell were calculated as a mean of 10 counts.

Nitroblue Tetrazolium Reduction (NBT)

The NBT reduction was assayed as previously described (7) with PMN and bacteria from the phagocytosis experiments. For the stimulation test, 0·5 ml of the saline dextrose solution was replaced by an equal volume of a suspension of the *N. gonorrhoeae* strain being tested. Percentage positive counts (i.e. cells containing formazan deposits) were made of the preparations and the mean of 10 counts compared statistically by a paired *t*-test with the unstimulated control cells performed at the same time.

Attachment Experiments

Blood from healthy donors with no history of gonorrhoea was collected in plastic tubes containing 10 units of heparin ml^{-1}. Monocytes and PMN were separated on Ficoll-Hypaque cushions (Ficoll, Pharmacia, London; specific gravity 1·078) by the method of Böyum (8). After the interface cells (monocytes and lymphocytes) were collected, the pellet containing granulocytes and red blood cells was resuspended in 0·8% ammonium chloride to lyse the erythrocytes (9). The separated cells were finally washed twice with Dulbecco's phosphate buffered saline (DPBS) pH 7·2. PMN were resuspended in Eagles' minimal essential medium (MEM) without serum, buffered with HEPES at pH 7·3 (final concentration, 30 mM; Wellcome Research Laboratories, Beckenham), and supplemented with penicillin/streptomycin (final concentration 100 μml^{-1} of each) and glutamine (final concentration 2 mM) to give a total cell count of 2×10^5 (3 ml). Aliquots (1 ml) of the above cell preparations were layered onto glass coverslips 13 mm diameter in tissue culture plates with wells of 16 mm diameter (Costar, 295 Broadway, Cambridge, Ma.) and incubated for 1 hr at 37°C to allow adhesion. Non-adherent cells were removed by repeated washing in DPBS.

Coverslips were overlaid with 1 ml of gonococci (5×10^8/ml) in DPBS containing Ca^{++} 0·9 mM and Mg^{++} 0·5 mM and incubated for (a) 3 hr at 4°C, or (b) 30 min at 37°C. Non-attached organisms were removed by repeated washing with DPBS. Coverslips were air-dried, fixed in methanol and stained with May Grünwald/Giemsa. Bacterial binding was estimated by counting leukocytes with bacteria attached at two or more discrete points. Duplicate coverslips were used; for each coverslip, 200 leukocytes were counted and the results expressed as the percentage of cells binding organisms. The percentages of leukocytes with 1, 2–3, 4–5, 6 or 6 bacteria per cell were also determined.

Fresh normal sera were obtained from the same healthy donors of blood groups A, B, O and AB from whom the leukocytes were isolated. A portion of each serum was inactivated at 56°C for 30 min. The effect of opsonization on attachment was assayed as above by preincubating the bacterial suspensions with 0·1 ml of the homologous heat-inactivated serum with 0·9 ml of bacteria in HBSS before they were added to the phagocytic cells.

Results

Isohaemagglutinins as Opsonins

Sera from individuals of blood groups A, B or O were used as a source of isohaemagglutinins; the control contained HBSS instead of serum. The titres of isohaemagglutinin were 256 for anti-A and anti-B in the B and A individuals respectively and 64 for anti-A and 32 for anti-

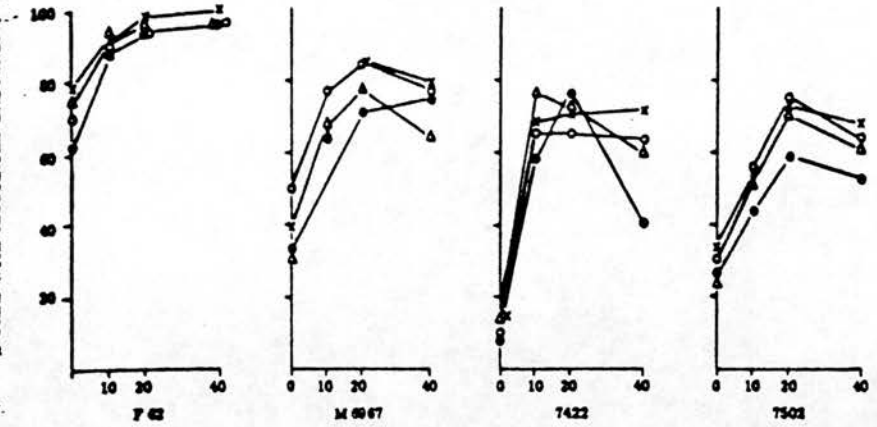


FIG. 1. Phagocytosis of *N. gonorrhoeae* strains opsonized with A, B, or O serum. ●: no serum, ○: A serum, △: B serum, X: O serum.

B in the O individual. Figure 1 shows the effect of these various sera on attachment and phagocytosis (i.e. association) of various strains of gonococci at 37°C.

The figure shows that association was independent of the presence of the isohaemagglutinins; the variations observed were dependent on strain differences. The strains included a laboratory strain (F62) subcultured for a number of years that showed the highest degree of association; a freshly isolated clinical strain (M6967); and two strains isolated from patients with disseminated gonococcal infection (7422 and 7502).

Attachment to PMN of Strains with Different Lipopolysaccharide Composition Determined by Pyocin Type

The strains used can be subdivided according to the complexity of their LPS as defined by sensitivity to R-type pyocins. Group I strains can be simply regarded as sensitive to the pyocins that attach to LPS structures nearest the cytoplasmic membrane, whereas group II strains are sensitive to the same pyocins as group I and also to additional ones that attach to LPS components in positions more distal from the cytoplasmic membrane. A clinical isolate of group I (E757) and one of group II (P152) were examined for their ability to attach to PMN from individuals of blood groups A, B, O and AB. At 4°C and 37°C (data not shown) in the absence of homologous serum, the group II organisms with the more complex LPS associated with larger numbers of PMN than the group I organism (fig. 2). The same differences in association were apparent between the two groups when the bacteria were preincubated with homologous heat-inactivated serum (data not shown).

The numbers of gonococci associating with individual PMN were determined for organisms of both groups (figs. 3 and 4). More bacteria of group II than group I were

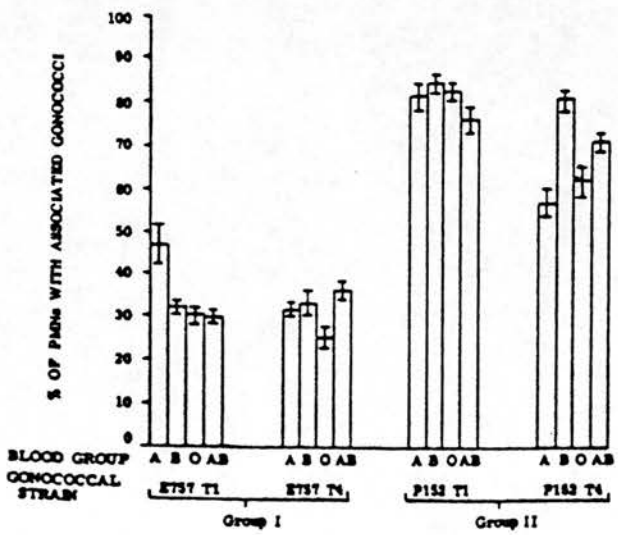


FIG. 2. Association of pilate and non-pilate variants with PMN from different blood groups at 4°C in the absence of serum.

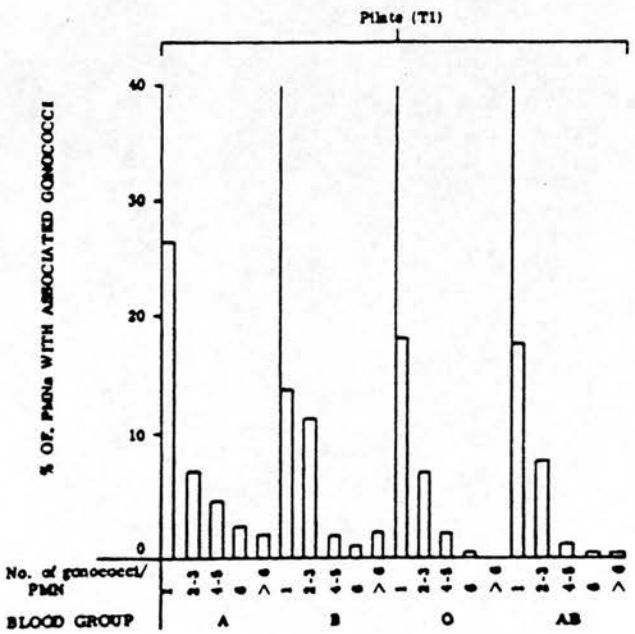


FIG. 3. Numbers of pilate gonococci of group I associated with individual PMN of different blood groups at 4°C in the absence of serum.

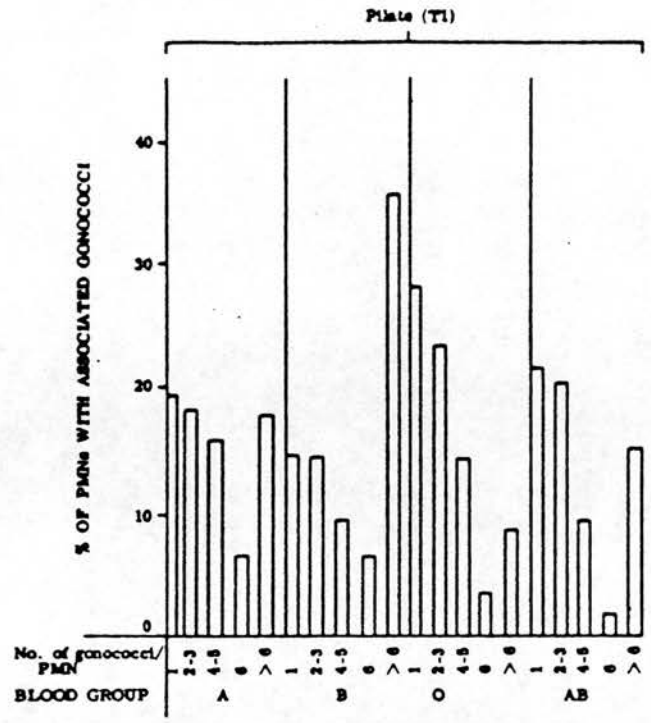


FIG. 4. Numbers of pilate gonococci of group II associated with individual PMN of different blood groups at 4°C in the absence of serum.

associated with individual PMN (fig. 3). Similar patterns were observed in the presence of homologous heat-inactivated serum at 4°C and 37°C, although fewer organisms of either group attached at 4°C.

Factors Affecting NBT Reduction

Pilate and non-pilate variants of group I and group II strains were assessed for their potential to stimulate NBT reductase in PMN. Two strains of group I (M9131 and E757) and 5 strains of group II (F62, M8865, M6967, E760 and 9) were used. No differences in the number of formazan-containing PMN were found in NBT tests with non-pilate variants of the two groups (table 2). In contrast, for each strain tested, the presence of pili resulted in a significantly lower number of PMN-containing formazan deposits compared to results obtained with its non-pilate variant (table 3).

Table 2 Stimulation of NBT reduction by non-pilate (T4) variants of gonococci of groups I and II (percentage PMN containing formazan)

Strain	Number of experiments	Mean percentage PMN containing formazan (SE) Unstimulated (control)	Stimulated (test)
Group I			
M9131	3	3·1 (0·62)	8·87 (2·8)
E757	2	3·8 (0·55)	24·2 (4)
Group II			
F62	3	5·9 (1·1)	24·03 (2·11)
M6967	2	8·4 (2·9)	22·4 (0·5)
9	2	3·9 (1·5)	13·9 (2·1)
M8865	3	3·1 (0·62)	19·3 (2·1)
E760	5	6·0 (2·2)	27·6 (6·6)

Table 3 The effect of pilation on NBT reduction with gonococci of group I and group II (percentage PMN containing formazan)

Strain	Number of experiments	Mean percentage PMN containing formazan (SE) Unstimulated (control)	Stimulated (test)
Group I			
E757 (T4) non-pilate	2	3·8 (0·55)	24·2 (4)*
E757 (T1) pilate	2	3·8 (0·55)	7 (4·5)†
Group II			
F62 (T4) non-pilate	1	10·6	25·9*
F62 (T1) pilate	1	10·6	10·6†
E760 (T4) non-pilate	5	6 (2·2)	27·6 (6·6)*
E760 (T1) pilate	5	6 (2·2)	11·3 (3)†

* $p < 0.001$.
† N.S.

DISCUSSION

We have tested the hypothesis proposed by Foster and Labrum (1) that the observed excess of blood group B individuals among patients with gonococcal infection may indicate that the anti-B isohaemagglutinin is a more effective opsonin than the anti-A isohaemagglutinin. We were unable to demonstrate any significant difference in the association of individual strains of *N. gonorrhoeae* opsonized with fresh sera from healthy A, B, O or AB individuals to PMN of the respective blood groups.

Our results indicate that differences in association of gonococcal strains with PMN from A, B, O or AB individuals was independent of the serum and paralleled differences in LPS composition as detected by the sensitivity of the strain to R-type pyocins of *P. aeruginosa* (10, 3, 11). The strain of group II gonococci with the more complex LPS was found to attach to a greater percentage of PMN than the strain of group I. The same pattern emerged when the numbers of gonococci per individual PMN were determined.

Differences in attachment of gonococci to PMN from donors of all four blood groups appears to depend on the LPS composition as determined by pyocin type, rather than the presence of pili or opsonization by anti-B antiserum.

In contrast to our observations on association with PMN, we found no differences in the NBT test with PMN when non-pilate strains of either group I or group II were tested. Both groups were found to show an increase in number of formazan-containing PMN compared with the control to which no bacteria were added. Results for pilate variants of both groups were not significantly different from the unstimulated controls.

Densen and Mandell (12) reported that in the presence of serum, pilate and non-pilate gonococci of their test strain F62 stimulated specific granule release measured by the appearance of lactoferrin in the medium, and that both stimulated increases in oxidative metabolism. In contrast, Kreiger, Schiller and Roberts (13) reported a minimal increase in glucose oxidation and oxygen consumption of PMN to which pilate gonococci were attached compared with the marked increase found with non-pilate gonococci. It must be noted that the latter workers were using a pilate strain and a non-pilate strain obtained from two different patients with disseminated infections. Our results in studies with pilate and non-pilate variants of strain F62 and with isolates from localized genital infections support the findings of Kreiger *et al.* and not those of Densen and Mandell.

We conclude that in host parasite interactions between PMN of the four different blood groups and *N. gonorrhoeae*, the surface characteristics of the bacteria detected by the pyocin typing system are the major determinants of recognition. These surface characteristics appear to have no effect on subsequent intracellular events that are linked with the NBT assay. Our findings support previous reports (13, 14) that gonococcal cells with pili, cell surface components associated with virulence, provoke less stimulation of intracellular bactericidal mechanisms in human PMN than do non-pilate organisms. Although antibodies against pilar proteins are produced during gonococcal infection (15), electron microscopic studies of infected material have prompted debate about their physical presence on the bacteria *in vivo* (16, 17). It is possible, however, that proteases present in the tissue fluids and released by phagocytes may remove these structures.

The greater ability of group II gonococci with more complex LPS to adhere to PMN would be expected to [illegible] to the disadvantage of the organism in establishing infection. This would, however, be offset by any advantage such organisms would have in attaching to uroepithelial cells. Group II organisms are frequently recovered from patients (3); thus, it will be necessary to determine if the differences in attachment to PMN observed for groups I and II are found also with uroepithelial cells. Pili are already known to play a part in mediating such attachment (18, 19) but, the relative contributions of pili and LPS are not yet established.

The present report has concentrated on gonococcal-PMN interactions. Novotny and colleagues (20) have proposed that gonorrhoea is a disease of human monocytes. Whether or not the presence of pili or differences in LPS have any significance as determinants of susceptibility in individuals of different blood groups has not yet been elucidated. As a result of the present study and our previous findings (4) that pili are an important determinant for attachment to monocytes, investigations are underway to examine various factors, of both host and parasite, affecting recognition of gonococcal strains by monocytes of different ABO blood groups.

ACKNOWLEDGEMENTS

We are grateful to Professor J. G. Collee for help in the preparation of the manuscript and to Mrs. Marilyn Cole for its typing. The work was supported by Grant No. K/MRS/50/C22 from the Biomedical Research Committee, Scottish Home and Health Department and by Grants G81/103811 SB and G979/64/S from the Medical Research Council.

REFERENCES

1. Foster, M. T. and Labrum, A. H. (1976). Relation of infection with *Neisseria gonorrhoeae* to blood groups. *J. Infect. Dis.*, 133, 329.
2. Miler, J. J., Novotny, P., Walker, P. D., Harris, J. R. W. and MacLennan, I. P. B. (1977). *Neisseria gonorrhoeae* and ABO isohaemagglutinins. *Infect and Immun.*, 15, 713.
3. Blackwell, C. C., Young, H. and Anderson, I. (1979). Sensitivity of *Neisseria gonorrhoeae* to partially purified R-type pyocines and a possible approach to epidemiological typing. *J. Med. Microbiol.*, 12, 321.
4. Kinane, D. F., Blackwell, C. C., Weir, D. M. and Winstanley, F. P. (1982). Phagocyte recognition of *Neisseria gonorrhoeae*. *J. Clin. Lab. Immunol.*, 9, 169.
5. Young, H., Patterson, L. C. and McDonald, D. R. (1976). Rapid carbohydrate utilization test for the identification of *Neissera gonorrhoeae*. *Brit. J. vener. Dis.*, 52, 172.
6. Van Furth, R., Van Zwet, T. L. and Leijh, P. C. J. (1978). *In vitro* determination of phagocytosis and intracellular killing by polymorphonuclear and mononuclear phagocytes. In *Handbook of Experimental Immunology*, (edited by D. M. Weir). Blackwell Scientific Publications, Oxford.
7. Kowolik, M. J. and Moody, G. H. (1979). A simplified technique for the NBT test. *IRCS Medical Science Cell and Molecular Biology*, 7, 100.
8. Böyum, A. (1968). Isolation of leucocytes from human blood. Further observation. Methyl cellulose dextran and Ficoll as erythrocyte aggregating agents. *J. Clin. Lab. Invest.*, 21, Suppl. 97, 31.
9. Boyle, W. (1968). An extension of the ^{51}Cr release assay for the estimation of mouse cytotoxins. *Transplantation*, 67, 761.
10. Sidberry, H. D. and Sadoff, J. C. (1977). Pyocin sensitivity of *Neisseria gonorrhoeae* and its feasibility as an epidemiological tool. *Infect and Immun.*, 15, 628.
11. Sadoff, J. C., Zollinger, W. D. and Sidberry, H. D. (1978). Cell surface structures of *Neisseria gonorrhoeae*. In *Immunobiology of* Neisseria gonorrhoeae, pp. 93–100. American Society for Microbiology, Washington D.C.
12. Densen, P. and Mandell, G. (1977). The role of microbial attachment and ingestion in polymorphoneutrophil function. *Clin. Res.*, 25, 375A.
13. Krieger, A. G., Schiller, N. L. and Roberts, R. B. (1980). Gonococci—human polymorphonuclear leucocyte interactions: metabolic studies associated with attachment and ingestion. *Infect. and Immun.*, 8, 991.

J. Clin. Lab. Immunol. (1983), 11, 27–32

BLOOD GROUPS AND SUSCEPTIBILITY TO GONOCOCCAL INFECTION.
II. THE RELATIONSHIP OF LIPOPOLYSACCHARIDE TYPE TO GONOCOCCAL SENSITIVITY TO THE BACTERICIDAL ACTIVITY OF NORMAL HUMAN SERUM

F. P. WINSTANLEY, C. C. BLACKWELL, D. M. WEIR and D. F. KINANE
Department of Bacteriology, University of Edinburgh Medical School, Teviot Place, Edinburgh EH8 9AG, UK
(Received 2 August 1982)

SUMMARY This study examines the bactericidal activity of normal human sera from individuals of blood groups A and B for gonococcal strains with simple and complex lipopolysaccharides (defined by pyocin-sensitivity) isolated from localised and disseminated infection. The bactericidal activity did not depend on A or B isohaemagglutinins. Resistance to normal human serum exhibited by strains from localised infections appeared to be due to lack of part of the lipopolysaccharide antigen, whereas resistance of strains from disseminated infection appeared to depend on a separate mechanism yet to be defined.

INTRODUCTION

INDIVIDUALS of blood group B are especially susceptible to genito-urinary infections with *Escherichia coli* (1, 2) and *Neisseria gonorrhoeae* (3, 4, 5). There is evidence that the anti-B isohaemagglutinin found in individuals of blood groups A and O may play a role in host-protection against infection by gram-negative bacteria (6).

In this study we investigated the sensitivity of a number of strains of *N. gonorrhoeae* with different lipopolysaccharide (LPS) to "normal human serum" (NHS) from individuals of blood groups A and B to determine whether anti-B isohaemagglutinin is involved in a non-specific host defence mechanism against gonorrhoea.

Previous work from this laboratory has indicated that attachment of gonococcal strains to polymorphonuclear leukocytes (PMN) varies according to differences in the structure of the LPS (7) as detected by differences in sensitivity to partially purified R-type pyocins of *Pseudomonas aeruginosa* (8). Since LPS is one of the major antigens involved in bactericidal killing (9, 10), strains of both of the pyocin-defined groups (7) were tested to determine whether differences in LPS structure could be correlated with differences in sensitivity to the bactericidal effects of NHS and conventional immune sera prepared in mice.

Correspondence: Dr. D. M. Weir, Department of Bacteriology University of Edinburgh Medical School, Teviot Place, Edinburgh, EH8 9AG.

MATERIALS AND METHODS

Bacterial Strains

Ten gonococcal strains from localised infections were supplied by the Department of Genito-urinary Medicine, Royal Infirmary of Edinburgh (R.I.E.). Three β-lactamase-producing strains were kindly supplied by Dr. K. Shannon and Professor I. Phillips, St. Thomas's Hospital Medical School, London, and two strains from disseminated infections were obtained from Dr. Joan S. Knapp, Neisseria Reference Laboratory, United States Public Health Service, Seattle, Washington. All strains, together with the grouping based on pyocin sensitivity (7) are listed in Table 1. They all conformed to the morphological and biochemical characteristics of *N. gonorrhoeae* and were of colony type 4 (11).

Culture

Strains were cultured on Modified New York City (MNYC) medium (12) at 37°C in a humidified incubator with 10% CO_2 in air.

Isolation of Pyocin-resistant Mutant

Colonies growing within a zone of inhibition produced by pyocin IS8 were isolated and re-typed. One mutant strain (P280μ), sensitive to Kageyama group R5 pyocins only, was used in the study.

Preparation of Immunising Antigens

An 18-hr growth of gonococci was harvested from MNYC plates with sterile cotton swabs and suspended in 0·01M phosphate-buffered saline (PBS) pH 7·2. An equal volume of PBS containing 2·4% formaldehyde was added to the suspension which was stored overnight at 4°C. The formaldehyde-treated organisms were centrifuged at 900 × g for 1 hr and resuspended in PBS containing 0·01% formaldehyde to the opacity of Brown's Tube No. 3 as judged by eye (13). This is equivalent to $1·1 \times 10^9$ gonococci/ml (14).

0141-2760/83/0500-0027 $04.50

Table 1 Pyocin type of gonococcal strains

		Pyocins with Kageyama group												
		R1					*Unclassified*		*R3*	*R2*			*R5*	
	Gonococcal strain	*1SD*	*2285*	*1S4*	*1S6*	*1S8*	*R205*	*9579*	*1SE*	*1SB*	*R21*	*430*	*1SA*	*1SC*
Group I	M9131													
	E757													
	*849209													
	*845650	−	−	−	−	−	−	+	−	−	−	−	+	+
	*934936													
	P280μ													
	P3309													
	†7425													
Group II	M8865	+	±	+	+	+	+	+	−	−	−	−	+	+
	P280													
	E2590													
	E728													
	E759	+	+	+	+	+	+	+	−	−	−	−	+	+
	M5287													
	P3290													
	†1560													

+ clear zone of inhibition.
± zone of partial inhibition.
− no inhibition.
* β-lactamase producing strains.
† strains from disseminated infection.

Immunisation Schedule

Female CF1 mice, 5–6 weeks old, caged in groups of 10, were given 0·25 ml of the standard suspension of formaldehyde-treated gonococci (gonococcal antigen) intravenously, via the lateral tail veins. The mice were immunised at day 0 and again on days 7 and 10. On day 14 a small quantity of blood was taken from the retro-orbital plexus of each mouse of the group and pooled: the serum obtained was inactivated by heating to 56°C for 30 min and then distributed into small tubes and stored at −20°C until required. The mice thereafter received booster doses (0·25 ml) of the gonococcal antigen at 14-day intervals and blood was obtained 5 days after each immunisation.

Complement

Human AB serum from a donor with no history of gonococcal infection was adsorbed for 24 hr at 4°C with a live suspension of the gonococci used in the study, distributed in 200 μl aliquots and stored at −70°C before use. The minimum haemolytic titre of the serum was 32–64.

Normal Human Sera from Individuals of Blood Groups A and B

Kindly supplied by Drs. S. Urbaniak and P. L. Yap of the Blood Transfusion Service, R.I.E.

Screening Test for Bactericidal Activity of Normal Human Serum

An 18-hr culture of gonococci was suspended in Dulbecco's Phosphate Buffered Saline supplemented with Mg^{++} and Ca^{++} ions (0·5 mM and 0·9 mM respectively) (DPBS + B) to give approximately 10^4 cfu/ml.

A series of two-fold dilutions, from 15 to 480, were made in a microtitre plate for each serum to be screened to give a final volume of 50 μl per dilution, 40 μl of gonococcal suspension and 10 μl of a two-fold dilution of the complement source were added to each well and incubated at 37°C for 30 min. Two drops of 20 μl from each well were plated onto MNYC medium and incubated for 24 hr. The highest serum dilution to give a reduction in viable count of ⩾ 80 % was considered to be the bactericidal titre of the serum.

Bactericidal Assay with Immune Mouse Serum

These were performed in round-bottomed glass tubes (15 mm × 75 mm). An 18-hr culture of gonococci was suspended in D.PBS + B to give approximately 10^6 cfu ml. The assay mixture contained 160 μl of bacterial suspension, 20 μl of a two-fold dilution of heat-inactivated mouse serum and 20 μl of freshly thawed AB serum as the complement source. Controls lacking serum and/or complement were included for each experiment. After static incubation at 37°C for 30 min in a humidified atmosphere with 10 % CO_2 in air, 25 μl volumes were removed from each tube and viable counts were performed (15). A reduction in viable count of 80 % or greater when compared with controls was considered to be significant.

Results

The gonococcal strains were all typed by pyocin sensitivity and classified into two broad groups: Group I strains were sensitive to Kageyama group R5 pyocine; Group II strains were sensitive to Kageyama groups R5 and R1 pyocins (table 1).

The AB serum used as the exogenous complement source for the bactericidal reactions was found to have slight microbicidal activity against strains of Group II. This activity was removed by absorption at 4°C with the sensitive strains and the procedure had no effect on the complement titres. Absorbed AB serum was used in all the bactericidal tests.

Table 2 Bactericidal titre of normal human serum from Group A individuals

	Test strain of N. gonorrhoeae	Sera A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P
Group I	M9131, E757, 849209, P3309, 934936, P280μ, *7425	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<
Group II	M8865	15	240	60	60	60	30	120	120	30	120	120	60	30	30	15	60
	P280	>	>	>	>	>	>	240	240	240	60	120	30	120	120	30	120
	E2590	<	<	60	30	<	30	<	<	30	30	120	60	60	60	15	60
	P3290	15	15	30	30	30	30	15	60	120	120	15	15	15	60	30	120
	*1560	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<

< less than 15.
> greater than 480.
* strains from disseminated infection.

Normal human sera from individuals of blood groups A and B were found to be bactericidal to gonococcal strains of Group II to a titre of ⩾ 15, but no activity towards Group I strains from localised infections or a disseminated infection could be demonstrated at the lowest serum dilution of 15. The titre of bactericidal antibody against strains of Group II was slightly greater in individuals of blood group A (tables 2 and 3). No bactericidal activity could be demonstrated towards strain 1560 (Group II) from a disseminated infection (tables 2 and 3).

This bactericidal activity of NHS towards strains of Group II is inhibited by 10 mM Mg-EGTA which sequesters Ca^{++} ions necessary for the classical pathway of complement activation.

There was no discrimination between strains of Group I and Group II by the microbicidal mechanism in immune mouse serum. Serum raised against a strain from Group I (M9131) killed other strains in Group I and also strains in Group II. This same lack of discrimination was shown by antiserum raised against a strain from Group II (M8865) (table 4). Similar experiments with two gonococcal strains from disseminated infections (1560 and 7425) showed no detectable microbicidal activity.

One serum from each of the blood groups A and B was absorbed twice for 24 hr at 4°C with a heavy suspension of erythrocytes expressing A or B antigens to remove the iso-haemagglutinins. This absorption did not remove any bactericidal activity from the sera. Absorption with a heat-killed suspension of *P. aeruginosa* strain ZD8 slightly reduced the bactericidal activity of the sera (table 5).

Table 3 Bactericidal titre of normal human serum from Group B individuals

	Test strain of N. gonorrhoeae	Sera i	ii	iii	iv	v	vi	vii	viii	ix	x	xi	xii	xiii	xiv	xv	xvi
Group I	M9131, E757, 849209, P3309, 934936, P280μ, *7245	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<
Group II	M8865	15	30	30	30	>	15	120	30	15	15	<	30	<	15	30	15
	P280	<	15	<	15	240	15	15	30	120	60	60	60	240	60	120	60
	E2590	<	<	<	15	60	<	<	<	30	30	30	60	30	60	30	30
	P3290	<	<	<	30	60	<	120	60	120	30	30	15	15	120	30	60
	*1560	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<

< titre less than 15.
> titre greater than 480.
* strains from disseminated infection.

Table 4 Microbicidal action of immune mouse serum. Number of tests out of 4 giving ⩾ 80 % kill

	Gonococcal strains	Antiserum	
		M9131 (group I)	*M8865 (group II)*
Group I	M9131	3	2
	E757	1	2
	849209	3	4
	845650	4	4
	934936	2	3
	P280μ	0	3
Group II	M8865	4	4
	M5287	4	4
	E728	3	2
	E759	2	2
	P280	1	4

Table 6 Relative positions of pyocin receptor sites on lipopolysaccharide (LPS) of *Pseudomonas aeruginosa*

*R3**	*R4**	*R2**	*R1**	*R5**
I	H	D	K	A
		F	L	B
		J	M	C
		U	N	E
			O	G
			P	
			Q	
(Sidberry and Sadoff 1977)			R	
			S	
			T	
ISE	–	ISB	ISD	ISC
		R21	IS4	ISA
		430	IS6	
			IS8	
(This study)			2285	

* Arranged in order of the receptor sites on the lipopolysaccharide (LPS) fraction of *P. aeruginosa* suggested by Kageyama (1975).
The receptor for R5 is nearest to, and that for R3 furthest from the cytoplasm.

Discussion

Our findings suggest that the increased susceptibility of individuals of blood group B to infection by *N. gonorrhoeae* is not related to differences in susceptibility of the organism to isohaemagglutinins. Although *N. gonorrhoeae* isolates have been shown to absorb anti-A and anti-B isohaemagglutinins (4) the bactericidal activity of normal human serum (NHS) could not be removed by absorbtion with red blood cells expressing A and B antigens.

Variations in the susceptibility of gonococci to the bactericidal effects of NHS have been reported (9, 16, 17). Although the LPS has been found to be the target antigen for "natural" antibody (16, 18), the particular components involved in the reaction had not been identified. Classification of gonococcal isolates by the sensitivity pattern to partially-purified R-type pyocins of *P. aeruginosa* has provided a method for detecting differences in LPS antigens. Clinical isolates of Group I which appear to have a simpler LPS structure are resistant to NHS while those with more complex LPS (Group II) are sensitive. The importance of LPS components in determining the reaction with NHS is illustrated by the change from sensitivity to resistance found in the mutant P280μ (Group I) derived from a NHS-sensitive clinical isolate, P280 (Group II). Alterations in the LPS associated with this mutation was shown by gel-electrophoresis and gas-liquid chromatography (unpublished data). These findings are similar to those of Morse and Apicella (19) for their gonococcal LPS mutant selected for resistance to pyocin 611–131.

A schematic representation of the relative positions of pyocin receptors on the LPS of *P. aeruginosa* proposed by (20) is shown for our range of pyocins and those used by Sidberry and Sadoff (21) in Table 6. The "natural" antibody of NHS appears to be directed against either: (1) the LPS structure(s) that form the receptor site(s) for pyocins of Kageyama's group R1; or (2) to one or more structures distal, but linked, to the receptor site for the R1 pyocins. Evidence for this second proposal comes from the work of Sadoff and co-workers (22). Gonococcal isolates were typed with pyocins from their collection and they found that the growth of serum-sensitive strains was inhibited by pyocins D, H and I. These three pyocins belong to Kageyama groups R2, R4 and R3 respectively which attach to LPS receptors distal to those for R1 (table 6). In contrast, our pyocins of these three groups

Table 5 Titres of bactericidal antibody before and after absorption

	N. gonorrhoeae strain	*Before absorbtion*	*Absorbed with erythrocytes*	*Absorbed with* P. aeruginosa
Group A serum (K)	P280	120	120	60
	M8865	120	120	60
	E2590	30	30–60	15
Group B serum (xi)	P280	120	120	15
	M8865	15	<	15
	E2590	15	15	<

had no activity against any of the gonococcal isolates tested (8).

The putative "natural" antibody responsible for the microbicidal activity of NHS differs in specificity compared with conventional immune sera raised in mice. While the "natural" antibody recognises determinants present only on organisms expressing the more complex LPS, the immune sera had a wider specificity and were bactericidal for strains of both Group I and Group II.

Previous work has suggested that resistance of gonococci to NHS is not due to the presence of masked antigens, blocking IgA antibodies, or deficiencies in complement function, but to the resistant organism's lack of an LPS determinant that binds the "natural" antibody (23); our findings support this view. The bactericidal activity of NHS towards strains of Group II could be absorbed in part by a heat-killed suspension of *P. aeruginosa* strain ZD8, suggesting that the activity may be due to "natural" antibody induced by commensal flora.

One implication of our findings is that the more NHS-sensitive Group II organisms should be restricted to localised infections and that Group I should predominate in disseminated infections. In an earlier study on pyocin types of gonococci from a number of geographic sources (8) 95 (86·4%) of 110 isolates from localised infections were of Group II compared with only 15 (13·6%) of Group I. In contrast, of the 24 isolates from disseminated infections, a higher percentage of Group I organisms (41·6%) were found. The proportion of isolates of Group II was 58·3%. Since organisms of Group II have been isolated from disseminated infections, simplicity of LPS does not appear to be the sole factor in determining the characteristics of a "disseminated" strain.

A Group II isolate from a disseminated infection was resistant to immune mouse serum in addition to NHS and this finding is the subject of further studies. This suggests that loss or masking of antigens can occur without interfering with the receptor sites for the R-type pyocins. This resistance to bactericidal antibody of a strain from disseminated infections was not found to be associated with anti-complementary activity of the organism.

We conclude that the bactericidal effect of NHS on strains of *N. gonorrhoeae* is mediated by antibodies that bind to a LPS portion associated with the binding site of R1 pyocins present in gonococci of Group II. Strains lacking this portion of LPS—(Group I)—are resistant to NHS. Thus "natural immunity" to gonorrhoea appears to be directed at organisms of Group II.

The basis for the increased susceptibility of blood group B individuals to gonorrhoea is still not established but seems not to be due to isohaemagglutinins. There is evidence in *E. coli* urinary tract infections that the anti-B titres are raised during infection with organisms expressing B-like antigens. This rise did not occur with anti-A isohaemagglutinins in response to A-like organisms (24). If this difference is reflected in other "natural antibody" responses of B individuals a possible basis for increased susceptibility to infection arises.

The sharing of antigens (galactose determinants) between the LPS of gonococci and blood group B antigen could also account for a diminished response in individuals of blood group B.

ACKNOWLEDGEMENTS

We are most grateful to Professor J. G. Collee for his advice in preparation of the manuscript and to Mrs. M. Cole and Mrs. N. Marr for its typing. This investigation was supported by grant K/MRS/50/C22 from the Biomedical Research Committee, Scottish Home and Health Department, and by grants G81/103811/SB and G979/63/S from The Medical Research Council.

REFERENCES

1. Cruz-Coke, R., Paredes, L. and Montenegro, A. (1965). Blood groups and urinary microorganisms. *J. Med. Genet.*, **2**, 185.
2. Kinane, D. F., Blackwell, C. C., Brettle, R. P., Weir, D. M., Winstanley, F. P. and Elton, R. H. (1982). ABO blood group, secretor state and susceptibility to recurrent urinary tract infection in women. *Br. Med. J.*, **285**, 3.
3. Foster, M. T. and Labrum, A. H. (1976). Relation of infection with *Neisseria gonorrhoeae* to ABO blood groups. *J. Inf. Dis.*, **133**, 329.
4. Miler, J. J., Novotny, P., Walker, P. D., Harris, J. R. W. and MacLennan, I. P. B. (1977). *Neisseria gonorrhoeae* and ABO isohaemagglutinins. *Infect. Immun.*, **15**, 713.
5. Kinane, D. F., Blackwell, C. C., Winstanley, F. P. and Weir, D. M. Blood group, secretor status and susceptibility to infection by *Neisseria gonorrhoeae*. *Br. J. Vener. Dis.* (in press).
6. Springer, G. F., Williamson, P. and Brindes, W. C. (1961). Blood group activity of gram-negative bacteria. *J. exp. Med.*, **113**, 1077.
7. Blackwell, C. C., Kowolik, M., Winstanley, F. P., Kinane, D. F., Weir, D. M., Law, J. A. and Ho Wang Chok, Y.-L. (1982). Blood group and susceptibility to gonococcal infection. I. Factors affecting phagocytosis of *Neisseria gonorrhoeae*, *J. Clin. Lab. Immunol.* (in press).
8. Blackwell, C. C., Young, H. and Anderson, I. (1978). Sensitivity of *Neisseria gonorrhoeae* to partially purified R-type pyocins and a possible approach to epidemiological typing. *J. Med. Microbiol.*, **12**, 321.
9. Glynn, A. A. and Ward, M. E. (1970). Nature and heterogeneity of the antigens of *Neisseria gonorrhoeae* involved in the serum bactericidal reaction. *Infect. Immun.*, **2**, 162.
10. Ward, M. E., Lambden, P. R., Heckels, J. E. and Watt, P. J. (1978). The surface properties of *Neisseria gonorrhoeae*: Determinants of susceptibility to antibody-complement killing. *J. Gen. Microbiol.*, **108**, 205.
11. Kellog, D. S. Jr., Peacock, W. L. Jr., Deacon, W. E., Brown, L. and Pirkle, C. I. (1963). *Neisseria gonorrhoeae* I. Virulence genetically linked to clonal variation. *J. Bacteriol.*, **85**, 1274.
12. Young, H. (1978). Cultural diagnosis of gonorrhoea with modified New York City (MNYC) medium. *Br. J. Ven. Dis.*, **54**, 36.
13. Brown, H. C. (1919–1920). Further observations on the standardisation of bacterial suspensions. *Ind. J. Med. Res.*, **7**, 238.
14. Cunningham, J. and Timothy, B. (1924). A comparison between the

numerical content of certain bacterial suspensions obtained by the haemocytometer method and Brown's opacity tubes. *Ind. J. Med. Res.*, **11**, 1253.

15. Miles, A. A., Misra, S. S. and Irwin, J. O. (1938). The estimation of the bactericidal power of the blood. *J. Hyg. Camb.*, **38**, 732.
16. Tramont, E. C., Sadoff, J. C. and Wilson, C. (1977). Variability of the lytic susceptibility of *Neisseria gonorrhoeae* to human sera. *J. Immunol.*, **118**, 1843.
17. Rice, P. A., McCormack, W. M. and Kasper, D. L. (1980). Natural serum bactericidal activity against *Neisseria gonorrhoeae* isolates from disseminated, locally invasive and uncomplicated disease. *J. Immunol.*, **124**, 2105.
18. Ward, M. E. and Glynn, A. A. (1971). Antibodies to gonococcal lipopolysaccharides in patients with gonorrhoea. *J. Med. Microbiol.*, **4**, p. ii.
19. Morse, S. A. and Apicella, M. (1982). Isolation of a lipopolysaccharide mutant of *Neisseria gonorrhoeae*—an analysis of the antigenic and biologic differences. *J. Inf. Dis.*, **145**, 206.
20. Kageyama, M. (1975). Bacteriocins and Bacteriophages in *Pseudomonas aeruginosa*. In *Microbial Drug Resistance: Report of the International Symposium on Bacterial Resistance* (edited by S. Mitsuhasi and H. Hashimoto), p. 291. University of Tokyo Press.
21. Sidberry, H. D. and Sadoff, J. C. (1977). Pyocin sensitivity of *Neisseria gonorrhoeae* and its feasibility as an epidemiological tool. *Infect. Immun.*, **15**, 628.
22. Sadoff, J. C., Zollinger, W. D. and Sidberry, H. (1978). Cell surface structures of *Neisseria gonorrhoeae*. In *Immunobiology of* Neisseria gonorrhoeae (edited by G. F. Brooks, E. C. Gotschlich, K. K. Holmes, W. D. Sawyer and F. E. Young), p. 93. American Society for Microbiology, Washington D.C.
23. Schneider, H., Griffiss, J. McC., Williams, G. D. and Pier, G. B. (1982). Immunological basis of serum resistance of *Neisseria gonorrhoeae*. *J. Gen. Microbiol.*, **128**, 13.
24. Drach, G. W., Reed, W. P. and Williams, R. C. (1971). Antigens common to human and bacterial cells: urinary tract pathogens. *J. Lab. Clin. Med.*, **78**, 725.

Teviot Scientific Publications Ltd
31 Montpelier Park
Edinburgh EH10 4LX, UK

J. Clin. Lab. Immunol. (1983), 12, 83–86

ABO BLOOD GROUPS AND SUSCEPTIBILITY TO GONOCOCCAL INFECTION. III. ROLE OF ISOHAEMAGGLUTININS IN INCREASED ASSOCIATION OF *NEISSERIA GONORRHOEAE* TO MONOCYTES FROM BLOOD GROUP B INDIVIDUALS

D. F. KINANE,[1] C. C. BLACKWELL,[1] D. M. WEIR,[1] F. P. WINSTANLEY[1] and R. A. ELTON[2]
[1]*Department of Bacteriology, University Medical School, Teviot Place, Edinburgh EH8 9AG and* [2]*Medical Computing and Statistics Unit, University Medical School, Teviot Place, Edinburgh EH8 9AG.*
(Received 22 March 1983)

SUMMARY The association of human monocytes of different ABO blood groups with gonococci was examined under various conditions. Blood group B monocytes in the presence of autologous heat inactivated serum (HIS) showed increased association over monocytes of the other ABO groups with their autologous sera. No statistically significant trends in ABO monocyte association were noted for assays carried out in the absence of autologous HIS. Absorption of isohaemagglutinins from sera reduced the gonococcus-monocyte association level to that of controls.

INTRODUCTION

INCREASED susceptibility to various infectious diseases has been linked to the ABO blood group of the individual (1–8). Correlation between increased susceptibility to infection with *Neisseria gonorrhoeae* and ABO blood group B has been noted in patients of different racial groups (5–7).

The underlying mechanisms of microbial pathogenicity involved in these host parasite interactions are not understood. Many bacteria cross-react with ABO blood groups (9–11). Miler and co-workers (6) have shown that infection with *N. gonorrhoeae* may reduce the titre of ABO blood group isohaemagglutinins and that gonococci *in vitro* were capable of absorbing both anti-A and anti-B isohaemagglutinins. This suggests that gonococci have both A and B-like antigens present in their cell wall. The possible protective role of isohaemagglutinins against these cross-reacting bacteria thus requires consideration. Anti-B isohaemagglutinin has been shown to be both bactericidal to (10) and a significant opsonin for (12) *E. coli* 086. Work in our laboratory, however, suggests that anti-A and anti-B isohaemagglutinins are not bactericidal for gonococci (13) and they did not result in significant differences in association with polymorphonuclear leucocytes (14). Further work has been carried out to evaluate the role of isohaemagglutinins in gonococcal interactions with monocytes of different blood groups.

MATERIALS AND METHODS

Bacterial Strains

Neisseria gonorrhoeae strains P152 and E757 recently isolated from patients attending the Department of Genitourinary Medicine were used throughout these experiments. P152 and E757 were of different pyocin sensitivities which suggests differences in their lipopolysaccharide (LPS) (15). E757 was of "simple" LPS type (Group I) whereas P152 was of "complex" LPS type (Group II) (14). Opaque colony types 2 (pilate) (T_2) and 3 (non-pilate) (T_3) were differentiated with a Zeiss stereoscope microscope with a double system of substage lighting and selectively subcultured and maintained on Difco GC base supplemented as described by Young (16) (GC agar). T_2 and T_3 growths, with more than 95% of the colonies stable, were employed. Bacteria were harvested from GC agar plates at 14–16 hr, suspended in Dulbecco's phosphate buffered saline (DPBS) by gentle pipetting, washed and finally resuspended gently in DPBS.

Bacterial concentrations were determined at E650 nm on a CE292 spectrophotometer (CECIL INSTRUMENTS, Cambridge, England) by reference to standards enumerated by microscope counts with a counting chamber. A concentration of 10^8 organisms/ml was used in all assays except those involving absorption of isohaemagglutinins where a concentration of 10^9 organisms/ml was used.

Phagocytes

Blood from healthy donors with no history of gonorrhoea was collected in plastic tubes containing 10 units of heparin ml^{-1}. Monocytes were separated on Ficoll–Hypaque cushions (FICOLL, PHARMACIA, London) specific gravity 1·078 according to the method of Boyum (17). After the interface cells (monocytes and lymphocytes) were collected, the separated cells were finally washed twice with DPBS.

Correspondence: Dr. C. C. Blackwell, Department of Bacteriology, University Medical School, Teviot Place, Edinburgh EH8 9AG.

0141-2760/83/1000-0083 $03.50

Sera

Autologous sera was obtained at the same time as the blood for the cells was taken. In some experiments heat inactivated sera (HIS) was used and this was obtained by inactivating sera in a water bath at 56°C for 30 min. Fresh human sera (FHS) and heat inactivated sera (HIS) were used at a final concentration of 10% in the appropriate well. The isohaemagglutinin levels of all sera were within normal limits.

Absorption of ABO Antibodies

The group O sera used for these experiments had both anti-A and anti-B titres equal to 256. The isohaemagglutinins anti-A and anti-B were absorbed by an equal mixture of A_1 and B washed RBC for 1 hr at 37°C and then overnight at 4°C. The absorption mixture of sera and RBC was approximately 50% (vol/vol). The antibody titres were rechecked after absorption and where necessary absorption was repeated until both anti-A and anti-B titres were reduced to zero. Haemagglutination tests to check original titres and titres after absorption were carried out as described by Miler (6).

Preparation of Monolayers

Monocytes were resuspended in Eagle's minimum essential medium (MEM) without serum buffered at pH 7·3 with HEPES (final concentration, 30 mM; WELLCOME RESEARCH LABORATORIES, Beckenham) and supplemented with penicillin streptomycin (final concentration 100 μ/ml^{-1} of each) and glutamine (final concentration, 2 mM) to give a total cell count of 2×10^5 ml^{-1}. Aliquots (1 ml) of the above cell preparations were layered onto 13 mm diameter (No. 1) glass coverslips in tissue culture plates 16 mm well diameter: (COSTAR, 295 Broadway, Cambridge, Mass.) and incubated for 1 hr at 37°C to allow adhesion. Non-adherent cells were removed by repeated washing with DPBS.

Binding Assay

Coverslips were overlaid with 1 ml of gonococci in DPBS containing Ca^{++} and Mg^{++} ions (0·9 mM and 0·5 mM, respectively) and incubated for: (1) 30 min at 37°C and (2) 2 hr at 4°C. In those experiments involving sera, 0·1 ml of the appropriate sera was added to the 0·9 ml of gonococci in DPBS. Non-attached organisms were removed by repeated washing with DPBS. Coverslips were air dried, fixed in methanol and stained with May Grunwald Giemsa. Bacterial binding was estimated by counting monocytes with bacteria attached at two or more discrete points. Duplicate coverslips were used: for each coverslip, 200 leucocytes were counted and the results expressed as the percentage of cells binding organisms.

Analysis of Results

Two factor analysis of variance was used to examine the effects of blood group and various conditions on gonococcal/monocyte association.

RESULTS

Figure 1 shows the association of gonococci to monocytes of different ABO blood groups under various conditions; Dulbecco's Phosphate Buffered Saline (DPBS) at 37°C for 30 min, DPBS at 4°C for 2 hr and autologous heat inactivated serum (HIS) at 37°C for 30 min. Each bar represents the mean of 3 experiments, different individuals were used for each experiment.

Two factor analysis of variance was used to examine the effects of blood group and of the various conditions on gonococcus-monocyte association for each strain separately.

A significant interaction in this analysis indicates that the effect of blood group on association varied between the different conditions. When DPBS 37°C and 4°C were compared in this way, no significant interaction was found for either strain, but when HIS was also included there was a significant interaction for both E757 ($p < 0·001$) and P152 ($p < 0·01$). As Figure 1 shows this is mainly due to the fact that association was greater for group B subjects when HIS was used whereas there was little difference in association between the blood groups when DPBS was used.

A similar analysis was carried out to examine the effects of blood group and strain on association for each condition separately. There was a significant interaction for HIS ($p < 0·05$) but not for DPBS 37°C or 4°C. In the

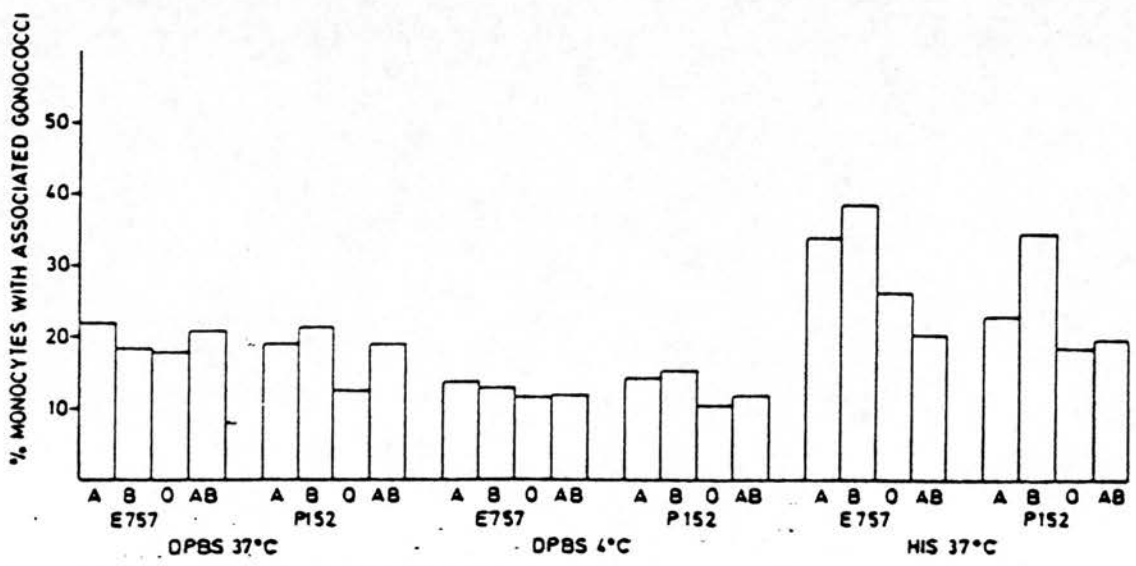


FIG. 1. Association of gonococci to monocytes of different ABO blood groups under various conditions. Each bar represents the mean of 3 experiments, different individuals were used for each experiment.

case of HIS, strain E757 in addition to showing the greater association with B monocytes shows more association with group A subjects than with those of groups O or AB, whereas strain P152, although likewise associating to a greater extent with B monocytes, gives little difference in association between the 3 other blood groups (fig. 1).

Thus LPS characteristics may not be important in gonococcus-monocyte association in DPBS but may be important when serum is present. Experiments using fresh human serum (FHS) were carried out but gave very low association levels for P152. We have noted previously in our laboratory that P152 was complement sensitive and suggest that it is this sensitivity to complement that gives the low association levels for P152 in FHS.

Comparing the association in HIS and DPBS at 37°C there is an overall increase in association for HIS throughout the blood groups with the exception of blood group AB. This is interesting bearing in mind that blood group AB homologous HIS contains no isohaemagglutinins in contrast to A, B and O homologous sera which do.

Figure 2 shows the effect of removal of isohaemagglutinins on gonococcus-monocyte association at 37°C. Group O monocytes and serum from different donors were used for each of 3 experiments. Absorption of isohaemagglutinins from HIS reduces binding levels to

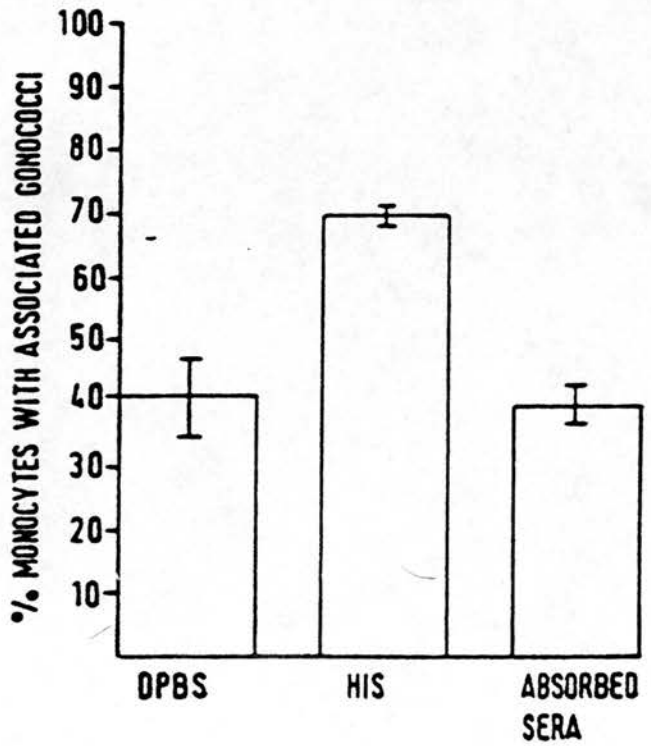


FIG. 2. The effect of removal of isohaemagglutinins on association of gonococci to monocytes of blood group O at 37°C. Each bar represents the mean ± 1 SEM of 3 experiments.

that of DPBS. It is likely therefore that although there are differences in binding patterns between strains, the significant interaction or binding pattern mentioned previously for HIS is due to the presence of isohaemagglutinins in the homologous sera.

DISCUSSION

Our results indicate that there are no differences in gonococcal association to monocytes of different ABO blood groups in the absence of autologous heat inactivated sera. This suggests that differences in ABO blood group antigen expression on monocytes does not influence gonococcal attachment (4°C) or phagocytosis and/or attachment (37°C). There were no significant differences between strains P152 and E757 in their binding in DPBS at both 4°C and 37°C. This contrasts with earlier work on PMN (14) which shows marked differences in binding between strains.

The association of gonococci to monocytes of different ABO blood groups in homologous heat inactivated sera (fig. 1) showed a significant pattern of binding for each strain, P152 ($p < 0.01$) and E757 ($p < 0.001$) with an increased level of association for group B monocytes. A further statistical analysis showed significant differences in pattern of association to different ABO monocytes for P152 and E757 ($p < 0.05$). Thus LPS characteristics seem to be important in gonococcus-monocytes association in autologous HIS. Heat inactivation was important, as strain P152 being complement sensitive, gave very low association levels when autologous fresh serum assays were carried out (not shown).

It is interesting to note that HIS increases the association for both strains for monocytes of all blood groups except AB, i.e. the blood group with no isohaemagglutinins. Miler (6) found that the isohaemagglutinins induced by gonococci were predominantly of the immunoglobulin M class as are isohaemagglutinins of normal sera. Haegart (18) has demonstrated the presence of IgM receptors on human peripheral monocytes. Our experiments indicate that isohaemagglutinins were responsible for the increased gonococcus-monocyte association over that found in DPBS. This differs from the findings for PMN where various isohaemagglutinins gave no significant increase (14).

These results would seem to contradict predictions that blood group B individuals may be more susceptible to the disease due to the ineffectiveness of their isohaemagglutinins in opsonizing bacteria for monocytes. In our previous report on phagocyte recognition of gonococci (19) we quoted "Novotny's hypothesis" (20) that gonorrhoea was a disease of human macrophages in which the gonococci attach to and enter macrophages where they multiply forming "infectious units" which go on to infect other sites. We noted increased binding of pilate gonococci to monocytes and suggested that this may be

virulence factor because increased affinity for monocytes would result in more "infectious unit" formation. In the light of these comments the results presented here suggest that group B individuals may be even more susceptible than individuals of other blood groups due to the increased association found for group B monocytes leading to the formation of more "infectious units".

ACKNOWLEDGEMENTS

We are most grateful to Professor J. G. Collee for his advice in preparation of the manuscript and to Mrs. M. Cole and Mrs. N. Marr for its typing. This investigation was supported by grant K/MRS/50/C22 from the Biomedical Research Committee, Scottish Home and Health Department and by grants G81/103811/SB and G979/63/S from The Medical Research Council.

REFERENCES

1. Barua, D. and Paguio, A. S. (1977). ABO blood groups and cholera. *Ann. Hum. Biol.*, **4**, 489.
2. Socha, W., Bilinska, M., Kaczera, Z., Pajdak, E. and Stankiewicz, D. (1969). *Escherichia coli* and ABO blood groups. *Folia. Biol. (Krakow)*, **17**, 259.
3. Robinson, M. G., Tolchrin, D. and Halpern, C. (1971). Enteric bacterial agents and the ABO blood groups. *Am. J. Hum. Genet.*, **23**, 135.
4. Cruz-Coke, R., Parades, L. and Montengro, A. (1965). Blood groups and urinary microorganisms. *J. Med. Genet.*, **2**, 185.
5. Foster, M. T. and Labrum, A. H. (1976). Relation of infection with *Neisseria gonorrhoeae* to blood groups. *J. Infect. Dis.*, **133**, 329.
6. Miler, J. J., Novotney, P., Walker, P. D., Harris, J. R. W. and MacLennan, I. P. B. (1977). *Neisseria gonorrhoeae* and ABO isohaemagglutinins. *Inf. Immun.*, **15**, 713.
7. Kinane, D. F., Blackwell, C. C., Winstanley, F. P. and Weir, D. M. (1983). Blood group secretor status and susceptibility to infection by *Neisseria gonorrhoeae*. *Br. J. Vener. Dis.*, **59**, 44.
8. Kinane, D. F., Blackwell, C. C., Brettle, R. P., Weir, D. M., Winstanley, F. P. and Elton, R. A. (1982). ABO blood groups, secretory sta u and susceptibility to recurrent urinary tract infection in women. *Br. Med. J.*, **285**, 7.
9. Springer, G. F. (1970). Importance of blood group substances in interactions between man and microbes. *Ann. N.Y. Acad. Sci.*, **169**, 134.
10. Muschel, L. and Osawa, E. (1959). Human blood group substance B and *Escherichia coli* 086. *Proc. Soc. Exp. Biol. Med.*, **101**, 614.
11. Drach, G. W. (1971). Antigens common to human and bacterial cells. Tolerance or resistance to urinary and surgical infections. *Surg. Forum*, **22**, 67.
12. Check, J. H., O'Neill, E. A., O'Neill, K. E. and Fuscaldo, K. E. (1972). Effect of anti-B antiserum on the phagocytosis of *Escherichia coli*. *Inf. Immun.*, **6**, 95.
13. Winstanley, F. P., Blackwell, C. C., Weir, D. M. and Kinane, D. F. (1983). Blood groups and susceptibility to gonococcal infection. II. The relationship of lipopolysaccharide type to gonococcal sensitivity to the bactericidal activity of normal human serum. In press.
14. Blackwell, C. C., Kowolik, M., Winstanley, F. P., Kinane, D. F., Weir, D. M., Law, J. A. and Ho Wang Chok, Y. L. (1983). ABO blood group and susceptibility to gonococcal infection. I. Factors affecting phagocytosis of *Neisseria gonorrhoeae*. *J. Clin. Lab. Immunol.*, **10**, 173.
15. Blackwell, C. C., Young, H. and Anderson, I. (1979). Sensitivity of *Neisseria gonorrhoeae* to partially purified R-type pyocines and a possible approach to epidemiological typing. *J. Med. Microb.*, **12**, 321.
16. Young, H., Patterson, I. C. and McDonald, D. R. (1976). Rapid carbohydrate utilisation test for the identification of *Neisseria gonorrhoeae*. *Br. J. Vener. Dis.*, **52**, 172.
17. Boyum, A. (1968). Isolation of leucocytes from human blood. Further observation. Methyl cellulose dextran and Ficoll as erythrocyte aggregating agents. *J. Clin. Lab. Invest.*, **21**, Suppl. 97, 31.
18. Haegart, D. G. (1979). Demonstration of IgM receptors on human peripheral monocytes using a direct plaque forming cell (PFC) assay. *J. Immunol. Meth.*, **28**, 97.
19. Kinane, D. F., Blackwell, C. C., Weir, D. M. and Winstanley, F. P. (1982). Phagocyte recognition of *Neisseria gonorrhoeae*. *J. Clin. Lab. Imm.*, **9**, 169.
20. Novotny, P., Short, J. A., Hughes, M., Miler, J. J., Syrett, C., Turner, W. H., Harris, J. R. W. and MacLennan, I. P. B. (1977). Studies on the mechanism of pathogenicity of *Neisseria gonorrhoeae*. *J. Med. Microb.*, **10**, 347.

Teviot Scientific Publications Ltd
31 Montpelier Park
Edinburgh EH10 4LX, UK

of chloracne (72). The table shows the mortality figures of both subgroups stratified by year of birth. 25 men have died, 8 from myocardial infarction including sudden death and 8 from neoplasms. In contrast to the 8 who died from tumours, 7 of the 8 men who died from myocardial infarction/sudden death had had chloracne; 4 belonged to the group of 16 contract cleaners, who did the dirtiest work and had severe chloracne. These 16 men had many other exposures to chemicals before and after the period concerned, and alcohol consumption was relatively high among this group. The neoplasms do not show an organ related pattern, and are not considered to represent any excess mortality: expected number of deaths 21 (S.MR 1·19, 95% confidence interval 0·78–1·70); expected number malignant diseases 6·94 (S.MR 1·15, 95% confidence interval 0·49–2·09).

Morbidity data are being collected on 35 of the cohort, most of them being employees of the factory concerned. The data are being compared with those of a matched control group, and to date no significant differences have been found. The study continues.

We thank Prof F. de Waard and Dr H. B. Bueno de Mesquita, National Institute for Public Health, Bilthoven, for statistical calculations.

Arbeidsinspectie, 1013 AE Amsterdam, Netherlands — L. M. DALDERUP

Duphar BV, Amsterdam — D. ZELLENRATH

GONORRHOEA, A PREDISPOSING FACTOR FOR MENINGOCOCCAL DISEASE?

SIR,—Immunity to meningococcal disease correlates with the presence of bactericidal antibodies for *Neisseria meningitidis*. Development of protective antibodies in the absence of meningococcal disease is associated with carriage of non-serogroupable strains of the organism which are also usually non-pathogenic.[1,2] Children not yet colonised by these strains are at greater than usual risk of meningococcal disease. We have noted abnormally low levels of antibody bactericidal for *N meningitidis* serogroup A and two non-serogroupable strains among individuals with gonorrhoea,[3] and report here the bactericidal activity of serum from individuals with gonorrhoea against strains of *N meningitidis* serogroups A, B, and C, those most frequently associated with meningococcal disease, and against the two non-serogroupable strains (0452 and NG846).

Strains of *N meningitidis* were cultured on modified New York City medium at 37°C in a humidified atmosphere enriched with 10% CO_2. Titration of bactericidal antibody was done as described previously (see table).[4]

The results demonstrate very low or undetectable levels of antibodies bactericidal for the strains of meningococci serogroups A, B, or C in sera from patients with gonorrhoea. This contrasts with the titres against a group A strain found in serum of uninfected "normal" individuals from the same geographical area (only 5/16 sera having titres below 4)[3] and with titres against serogroups A, B, and C reported by Craven et al.[5]

Absence of bactericidal antibody against serogroupable meningococci is a common feature of individuals who contract meningococcal disease and, as Artenstein and Ellis[6] suggested, "since meningococcal disease is a rare event compared to the carrier state, it may well be that deficiencies in host defences may play a major role in determining whether systemic invasion occurs".

1. Goldschneider I, Gotschlich EC, Artenstein MS. Human immunity to the meningococcus II: Development of natural immunity. *J Exp Med* 1969; 129: 1327–48.
2. Reller LB, MacGregor RB, Beaty HN. Bactericidal antibody after colonization with *Neisseria meningitidis*. *J Infect Dis* 1973; 127: 56–62.
3. Blackwell CC, Winstanley FP, Weir DM. Lipopolysaccharide type and sensitivity of the pathogenic *Neisseria* to the bactericidal activity of normal human serum. In: Proceeding of the fifth international conference on cerebrospinal meningitis (Marseilles, France, 1983).
4. Winstanley FP, Blackwell CC, Weir DM, Kinane DF. ABO blod groups and susceptibility to gonorrhoea II: The relationship of lipopolysaccharide type to gonococcal sensitivity to the bactericidal activity of normal human serum. *J Clin Lab Immunol* 1983; 11: 27–32.
5. Craven DE, Shea KT, Frasch CE. Natural bactericidal activity of human serum against *Neisseria meningitidis* isolates of different serogroups and serotypes. *Infect Immun* 1982; 37: 132–37.
6. Artenstein MS, Ellis RE. The risk of exposure to a patient with meningococcal meningitis. *Milit Med* 1968; 133: 474–77.

TITRE OF BACTERICIDAL ANTIBODY AGAINST N MENINGITIDIS STRAINS OF SEROGROUP A, B, AND C, AND TWO NON-GROUPABLE STRAINS 0452 AND NG846 IN SERA FROM PATIENTS WITH GONORRHOEA

Patient	A	B	C	0452	NG846
1	<4	8–16	4–8	32–64	16–32
2	<4	<4	8–16	64–128	<4
3	<4	8–16	8–16	64–128	8–16
4	<4	<4	<4	16–32	<4
5	8–16	8–16	16–32	64–128	32–64
6	<4	<4	8–16	≥128	64–128
7	<4	<4	<4	≥128	4–8
8	<4	<4	<4	64–128	16–32
9	<4	<4	<4	16–32	<4
10	<4	<4	<4	64–128	4–8
11	<4	<4	4–8	≥128	<4
12	<4	<4	4–8	≥128	128
13	32–64	<4	4	≥128	≥128
14	<4	<4	<4	≥128	8–16
15	≤4	<4	8–16	64–128	64–129
16	4	4	4–8	≥128	32–64
17	≥128	<4	4–8	≥128	≥128
18	4–8	<4	4–8	≥128	≥128

Meningococci and gonococci possess similar lipopolysaccharide structures,[7] and cross-reactions of monoclonal antibodies have been shown between lipopolysaccharide antigens of meningococci and gonococci.[8] Bactericidal antibodies against both these species appear to be directed against lipopolysaccharide antigens. The low titre of bactericidal antibody towards groups A, B, and C meningococci in sera from patients with gonorrhoea could be due to absorption of cross-reactive antibodies by the infecting strain of *N gonorrhoeae*.

We propose that gonorrhoea may compromise host defences against meningococcal disease and that this may explain in part the following observations:

(*a*) The age range most at risk of meningococcal disease is 0–4 years (ie, children lacking bactericidal antibodies[1]) but the group next most at risk is 15–19 years, the age when sexual activity begins and when the incidence of gonorrhoea is high.[9,10]

(*b*) During outbreaks of meningococcal disease in military establishments most patients are aged 18–24 years,[11,12] the range in which most cases of gonorrhoea in servicemen are found.

(*c*) The incidence of meningococcal colonisation of the pharynx is higher in patients with genital gonorrhoea (26%) than in those without (11%). The relative risk of harbouring meningococci when infected with gonococci is 2·44 (95% confidence limits, 1·4–4·2).[13,14]

A recent history of gonoccal infection or exposure to infection may be one of the contributing factors to the increased susceptibility of young adults to meningococcal disease.

We thank Dr H. Young and the staff of the STD diagnosis laboratory, department of bacteriology, for supplying sera and Mrs M. Cole for help in preparation of the report. The work was supported by grants from the Medical Research Council.

Department of Bacteriology, Medical School, University of Edinburgh, Edinburgh EH8 9AG — F. P. WINSTANLEY, C. C. BLACKWELL, D. M. WEIR, D. F. KINANE

7. Blackwell CC, Law JA. Typing of non-serogroupable *Neisseria meningitidis* by means of sensitivity to R-type procins of *Pseudomonas aeruginosa*. *J Infect* 1981; 3: 370–78.
8. Apicella MA, Bennett KM, Hermerath CA, Roberts DE. Monoclonal antibody analysis of lipopolysaccharides from *Neisseria gonorrhoeae* and *Neisseria meningitidis*. *Infect Immun* 1981; 34: 751–56.
9. Peltola H, Kataja JM, Makela PH. Shift in the age distribution of meningococcal disease as predictor of an epidemic? *Lancet* 1982; ii: 595–97.
10. Kallings LO, Moberg I. Epidemiology of gonorrhoea. In Skinner FH, Walker PD, Smith H, eds. Gonorrhoea, epidemiology and pathogenesis: FEMS symposium no. 2, London: Academic Press, 1977.
11. Varela GE, Gilmore GD. Meningococcal disease outbreak at Fort Leonard Wood, Missouri. *Milit Med* 1971; 136: 723–27.
12. Bristow WM, van Peenen PFD, Volk R. Epidemic meningitis in naval recruits. *Am J Publ Health* 1965; 55: 1039–45.
13. Young H, Harris AB, Robertson DHH. Individual susceptibility to venereal infection? *Br J Vener Dis* 1979; 55: 188–90.
14. Odegaard K, Gedde-Dahl TW. Frequency of simultaneous carriage of *Neisseria gonorrhoeae* and *Neisseria meningitidis*. *Br J Vener Dis* 1979; 55: 334–35.

Tissue Culture and RES
Eds: P. Röhlich and E. Bácsy (1984)

THE ROLE OF THE MONOCYTE IN GONOCOCCAL INFECTION

C.C. BLACKWELL, D.F. KINANE, F.P. WINSTANLEY, M.J. KOWOLIK and D.M. WEIR

Department of Bacteriology, University of Edinburgh Medical School, and Department of Oral Medicine and Oral Pathology, School of Dental Surgery, University of Edinburgh, Teviot Place, Edinburgh, Scotland

Studies on the pathogenicity of gonorrhoea have been severely limited by the lack of an experimental model since the causative organism, Neisseria gonorrhoeae, has only humans as its host. Epidemiological studies have been hampered by lack of a typing system for the bacteria as well as the social problems attached to contact tracing. One of the few relatively easily investigated host factors associated with susceptibility to gonorrhoea is the increased incidence of blood group B individuals among those infected (1,2). Even in an area such as Edinburgh where there is a high incidence of blood group B (10%) we found that more than 20% of gonorrhoea patients were blood group B (3).

Two hypotheses have been proposed to explain this increased susceptibility: (I) As with type 0:86 strain of Escherichia coli (4), anti-B isohaemagglutinin of groups A and O is a "natural" bactericidal antibody for some strains of gonococci; OR (II) Anti-B isohaemagglutinin of groups A and O is a more effective opsonin for some strains of gonococci (1).

Although gonococci had been shown to absorb isohaemagglutinins (2), we found that the natural antibody directed against them in normal human serum was not associated with either the anti-A or anti-B isohaemagglutinin. Bactericidal activity was found in all the sera tested. Killing depended on the lipopolysaccharide (LPS) structure of the bacteria : those with a complex LPS were serum sensitive, those with a simple LPS were serum resistant (5).

Having eliminated the first hypothesis, we examined the second - the role of isohaemagglutinins as opsonins. Based on their observed increase in the proportion of blood group B among women with gonorrhoea, Foster and Labrum suggested that the anti-B isohaemagglutinin may be a more effective opsonin than anti-A (1). We opsonized different strains of gonococci with serum from A, O and B individuals with no history of gonococcal infection and determined the percentage of bacteria bound to the polymorphonuclear leukocytes (PMN) of a group O donor.

There was no significant difference in association of PMN with gonococci opsonized by anti-A or anti-B isohaemagglutinin; however, as in the experiment screening for serum bactericidal activity, difference in association depended on LPS type. Those with the complex LPS were bound in greater numbers than those with the simple LPS to the PMN of each of the ABO blood groups in the absence or presence of the autologous serum.

We examined strains with the two LPS types for differences in stimulation of bactericidal activity with the nitroblue tetrazolium (NBT) test. Non-pilate strains of both LPS types significantly stimulated the NBT reaction as measured by the increase in the percentage of PMN containing the reduced formazan. Pilate variants of the same strains inhibited the NBT reaction. The percentage of formazan containing PMN did not rise above the control in which no bacteria were present (Table 1) (6).

Table 1 : The effect of pilation on NBT reduction with gonococci of group I and group II (percentage PMN containing formazan).

Strain	Number of experiments	Mean percentage PMN containing formazan (SE)	
		unstimulated (control)	stimulated (test)
Simple LPS			
E757 non-pilate	2	3.8 (0.55)	24.2 (4)*
E757 pilate	2	3.8 (0.55)	6 (4.5)+
Complex LPS			
F62 non-pilate	1	10.6	25.9*
F62 pilate	1	10.6	10.6+
E760 non-pilate	5	6 (2.2)	27.6 (6.6)*
E760 pilate	5	6 (2.2)	11.3 (3)+

* = $P < 0.001$

+ = N.S.

Similar association studies were carried out with strains of gonococci of simple and complex LPS and peripheral blood monocytes of groups A,B,O and AB.

In the absence of autologous heat inactivated serum (HIS), there were no significant differences in gonococcal association to monocytes of different ABO blood groups at 4° and 37°. There were no significant differences between simple and complex LPS types in contrast to our findings with PMN. This association of gonococci with monocytes is inhibited by sugars; therefore, it is probably mediated by the lectin-like receptor described by Weir and co-workers (7). (Fig.1.)

In the presence of HIS, there was an increase in binding of both strains to monocytes of A, B and O, but not AB, the blood group with no isohaemagglutinins. The greatest increase was observed for the group B monocytes. Absorption of O serum with A and B cells reduced the association to the level observed in the absence of serum (8), (Fig.2.)

Figure 1. Association of gonococci to monocytes of different ABO blood groups under various conditions.

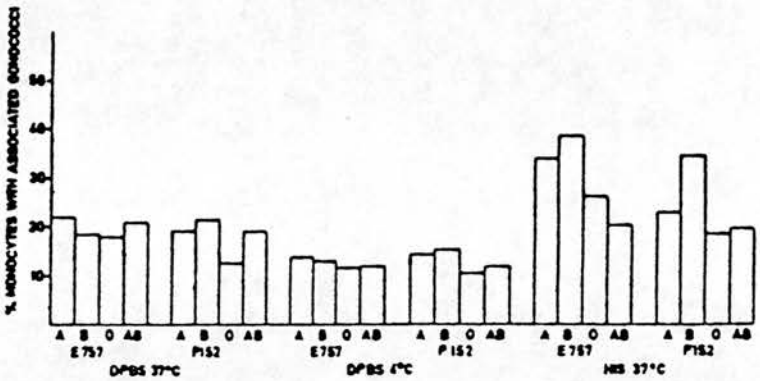
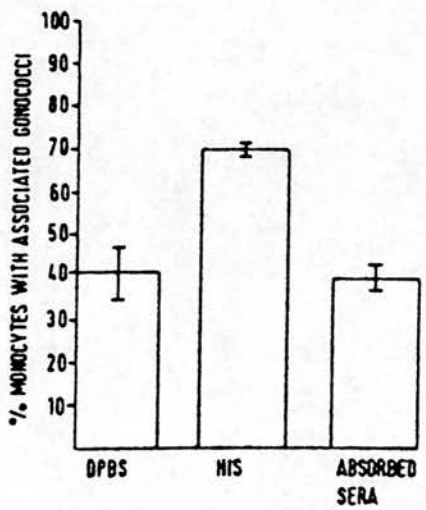


Figure 2. The effect of removal of isohaemagglutinins on association of gonococci to monocytes of blood group 0 at 37°C.



We have recently done some preliminary experiments to determine NBT reduction in monocytes exposed to N. gonorrhoeae, Neisseria meningitidis and Neisseria lactamica. The results shown in Table 2 indicate that opsonization of all three species with the fresh autologous serum from the monocyte donor lowered the percentage of NBT positive cells in comparison with results obtained with unopsonized cells. In contrast to our findings with PMN, pilate forms stimulated NBT reduction in monocytes, and the stimulation was greater than that of the non-pilate variant of the same strain.

References

Foster MT and Labrum AH. 1976. Relation of infection with Neisseria gonorrhoea to blood groups. J. Infect. Dis. 133 : 329.

Miler JJ, Novotny P, Walker PD, Harris JRW and MacLennan IPB. 1977. Neisseria gonorrhoea and ABO isohaemagglutinins. Infect. Immun. 15 : 713.

Kinane DF, Blackwell CC, Winstanley FP and Weir DM. 1983. Blood group, secretor status and susceptibility to infection by Neisseria gonorrhoeae. Br. J. Vener. Dis. 59 : 44.

Springer GF. 1970. Importance of blood group substances in interactions between man and microbes. Ann. N.Y. Acad. Sci. 169 : 134.

Winstanley FP, Blackwell CC, Weir DM and Kinane DF. ABO blood groups and susceptibility to gonococcal infection II. The relationship of lipopolysaccharide type to gonococcal sensitivity to the bactericidal activity of normal human serum. J. Clin. Lab. Immunol. (in press).

Blackwell CC. Kowolik MJ, Winstanley FP, Kinane DF , Weir DM, Law JA and Ho Wang Chok YL. ABO blood group and susceptibility to gonococcal infection. I. Factors affecting phagocytosis of Neisseria gonorrhoeae. J. Lab. Clin. Immunol. (in press).

Weir DM. 1980. Surface carbohydrates and lectins in cellular recognition. Imm. Today, 1, 45-51.

Kinane DF, Blackwell CC, Weir DM, and Winstanley FP. ABO blood groups and susceptibility to gonococcal infection. III. Role of isohaemagglutinins in increased association of Neisseria gonorrhoeae to monocytes from blood group B individuals. J. Clin. Lab. Immunol. (in press).

Novotny P, Short JA, Hughes M, Miler JJ, Syrett C, Turner WH, Harris JRW and MacLennan IPB. 1977. Studies on the mechanisms of pathogenicity of Neisseria gonorrhoeae. J. Med. Microbiol. 10 : 346-365.

J. MED. MICROBIOL. — VOL. 17 (1984), 353–356

SHORT ARTICLE

ABSENCE OF BACTERICIDAL ANTIBODIES AGAINST GROUP-I LIPOPOLYSACCHARIDE DETERMINANTS OF *NEISSERIA GONORRHOEAE* DURING HUMAN INFECTION

C. CAROLINE BLACKWELL, F. P. WINSTANLEY, D. M. WEIR AND D. F. KINANE

Department of Bacteriology, University of Edinburgh Medical School, Teviot Place, Edinburgh EH8 9AG

SUMMARY. None of 34 sera from patients with gonorrhoea contained antibodies bactericidal for strains of *Neisseria gonorrhoeae* with Group-I lipopolysaccharide (LPS). All contained antibodies against a strain with Group-II LPS, as do sera from uninfected controls. The absence of Group I-LPS antibodies in infected humans contrasts with previous findings that mice immunised with strains from either of the LPS groups produced bactericidal antibody to Group I. Our hope that detection of antibodies to Group-I strains would provide a screening test for gonorrhoea was, therefore, not realised.

INTRODUCTION

Gonococcal isolates from uncomplicated genital infections and disseminated infections have been divided into two broad groups based upon their pyocin-sensitivity spectrum. This classification has been shown to correlate with interactions of strains from localised genital infections with both humoral and phagocytic components of the human immune system. Briefly, gonococcal strains sensitive to pyocins of Kageyama's group R5, lipopolysaccharide (LPS) Group I, are resistant to normal human serum and they bind in lower numbers to polymorphonuclear leukocytes than strains of Group II (Blackwell *et al.*, 1983). This second group is sensitive to pyocins of Kageyama's group R1 in addition to R5 and strains of this group are killed by the majority of normal human sera tested (Kageyama, 1975; Blackwell, Young and Anderson, 1979; Winstanley *et al.*, 1983). Strains of Group II are thought to have extra LPS components, absent or cryptic in strains of Group I, to which there are bactericidal antibodies in the majority of normal human sera (Schneider *et al.*, 1982; Winstanley *et al.*, 1983).

Earlier studies revealed that specific antisera raised in mice against strains of Group I or II were bactericidal for other strains of the homologous group. They were also active against strains of the heterologous group (Winstanley *et al.*, 1983). In that study we also screened sera from a control population of women attending a postnatal clinic for bactericidal antibodies to both groups of gonococci. Antibodies against Group II only were found. This is a population in which there is a very low incidence of gonorrhoea. We have now screened sera from individuals with gonorrhoea for bactericidal activity against strains of LPS Groups I and II. Our hypothesis was that individuals exposed to or infected with *N. gonorrhoeae* would, like the mouse model, have antibodies to both groups and that detection of bactericidal activity for Group I might be used as a serological screening test for gonococcal infection.

MATERIALS AND METHODS

Bacteria. Strains of LPS Group I (E757 and M9131) and Group II (P280) were isolated from patients attending the Department of Genitourinary Medicine, Royal Infirmary, Edinburgh. All were from localised genital infections (Winstanley *et al.*, 1983). They were

Received 5 Sep. 1983; *revised version accepted* 2 Dec. 1983.

and uninfected patients; titres were $\leqslant 4$. The titres to strain P280 were similar to those recorded in the table.

Discussion

The absence or extremely low titres of bactericidal antibodies against strains M9131 and E757 in sera from patients with gonorrhoea refutes our hypothesis, based on results obtained with immune mouse sera (Winstanley *et al.*, 1983), that infection with *N. gonorrhoeae* in man would result in the production of antibodies bactericidal for strains of Group I. This failure may result from the stimulation by Group-I antigens of antibodies of classes that do not activate the classical complement pathway, e.g., classes IgA and IgG4, or by failure of the human immune system to respond to antigens of the gonococcal LPS associated with the binding site for R5 pyocins.

It has been suggested recently that susceptibility and resistance to gonorrhoea of a group of Chinese prostitutes was associated with differences in the major histocompatibility complex genes. The HLA "haplotype" A11, B15 appeared to be associated with resistance to the disease amongst these women who were at very high risk of exposure to infection (Chan and Rajan, 1982). Similarly, the ability to respond immunologically to LPS antigens associated with the binding site of R5 pyocins might depend upon the genetic constitution of the individual.

Bactericidal antibody is an important host defence that prevents the dissemination of gonococci from the mucosa (Brooks, Ingwer and Peterson, 1978). The majority (*c.* 85%) of gonococcal strains isolated from localised genital infection are of Group II (Winstanley *et al.*, 1983) and are thought to be prevented from dissemination by "natural" bactericidal antibodies and complement. Strains of Group I, however, being resistant to normal human serum, have a greater predisposition to cause bacteraemia; they are isolated from approximately 42% of disseminated infections (Winstanley *et al.*, 1983).

The apparent inability of man to produce antibodies bactericidal for gonococcal strains of Group I may be a host factor that retards recovery from disseminated gonococcal infection and it may also pose difficulties in the immunoprophylaxis of gonorrhoea. These results indicate that, like other attempts to identify antibodies against gonococcal LPS (Ward and Glynn, 1971 and 1972; Watt, Ward and Glynn, 1971; Maeland and Matre, 1975), screening of sera for bactericidal activity against LPS-group-I strains will not be of use as a diagnostic test.

We are grateful to Dr H. Young and the staff of the Serology Laboratory, Department of Bacteriology, for supplying us with sera and to Mrs M. Cole for help in preparation of the manuscript. The work was supported by grants G81/103811/SB and G979/64/S from the Medical Research Council. Mr D.F. Kinane was a recipient of the Davidson Research Fellowship, Faculty of Medicine, University of Edinburgh.

REFERENCES

Blackwell C C, Kowolik M J, Winstanley F P, Kinane D F, Weir D M, Law J A, Chok Y L H W 1983 ABO blood group and susceptibility to gonococcal infection I. Factors affecting phagocytosis of *Neisseria gonorrhoeae*. Journal of Clinical and Laboratory Immunology 10:173-178.

Blackwell C C, Young H, Anderson I 1979 Sensitivity of *Neisseria gonorrhoeae* to partially purified R-type pyocines and a possible approach to epidemiological typing. Journal of Medical Microbiology 12:321-335.

Brooks G F, Ingwer I, Peterson B H 1978 Importance of serum bactericidal and opsonic activity and complement in *Neisseria gonorrhoeae* infections. In: Brooks GF Gotschlich EC Holmes KK Sawyer WD Young FE (eds) Immunobiology of *Neisseria gonorrhoeae*. American Society for Microbiology, Washington, DC, pp 199-203.

Chan S H, Rajan V S 1982 HLA—resistance and susceptibility to venereal disease. In: Dawkins RL Christiansen FT Zilko PJ (eds) Immunogenetics in rheumatology, Excerpta Medica, International Congress Series No 602, Amsterdam, p 20.

Kageyama M 1975 Bacteriocins and bacteriophages in *Pseudomonas aeruginosa*. In: Mitsuhasi S

Hashimoto H (eds) Microbial drug resistance. University of Tokyo Press, Tokyo, pp 291-305.

Maeland J A, Matre R 1975 Specificity of human serum antibodies against endotoxin from *Neisseria gonorrhoeae*. British Journal of Venereal Diseases 51:176-178.

Schneider H, Griffiss J M, Williams G D, Pier G B 1982 Immunological basis of serum resistance of *Neisseria gonorrhoeae*. Journal of General Microbiology 128:13-22.

Ward M E, Glynn A A 1971 Antibodies to gonococcal lipopolysaccharides in patients with gonorrhoea. Journal of Medical Microbiology 4: p ii.

Ward M E, Glynn A A 1972 Human antibody response to lipopolysaccharides from *Neisseria gonorrhoeae*. Journal of Clinical Pathology 25:56-59.

Watt P J, Ward M E, Glynn A A 1971 A comparison of serological tests for the diagnosis of gonorrhoea. British Journal of Venereal Diseases 47:448-451.

Winstanley F P, Blackwell C C, Weir D M, Kinane D F 1983 Blood groups and susceptibility to gonococcal infection. II. The relationship of lipopolysaccharide type to gonococcal sensitivity to the bactericidal activity of normal human serum. Journal of Clinical and Laboratory Immunology 11:27-32.

Young H 1978 Cultural diagnosis of gonorrhoea with modified New York City (MNYC) medium. British Journal of Venereal Diseases 54:36-40.

J. Clin. Lab. Immunol. (1984), 13, 107–110

BINDING OF *NEISSERIA GONORRHOEAE* BY LECTIN-LIKE RECEPTORS ON HUMAN PHAGOCYTES

D. F. KINANE, D. M. WEIR, C. C. BLACKWELL and F. P. WINSTANLEY
Department of Bacteriology, University of Edinburgh Medical School, Teviot Place, Edinburgh EH8 9AG, UK

(Received 2 September 1983)

SUMMARY The adherence of gonococci to phagocytes was examined for the presence of "lectin-like" receptors capable of binding these bacteria. The data suggests such "lectin-like" receptors are present on PMN and monocytes that recognize sugars present in the gonococcal cell wall and that there are quantitative and qualitative differences in expression of these receptors. These findings may have importance in investigation of the interaction of gonococci with phagocytes and with the subsequent induction of immunity in the disease.

INTRODUCTION

ATTACHMENT of *Neisseria gonorrhoeae* to mucosal cells and their subsequent invasion are the initial steps in the establishment of gonococcal infection. Once the organisms have penetrated the mucosal barriers, their survival largely depends on their interaction with phagocytic cells. Attachment and uptake by polymorphonuclear leucocytes (PMN) may lead to destruction of the organism. Attachment to macrophages is likely to lead to initiation of an immune response by presentation of microbial antigen to the lymphoid cells. The interaction between phagocytes and bacteria is clearly an important determinant in the pathogenesis of the disease. Binding to the phagocyte membrane is dependent on the presence of appropriate adhesin-receptor interactions between bacteria and phagocytes.

Interaction between gonococci and PMN or monocytes may be mediated by lectins present either on the bacterium or the leukocyte. These lectins, or sugar-binding proteins, can be demonstrated by inhibition of binding reactions after preincubation of cells or bacteria with sugars. Various workers have attempted to demonstrate lectins on gonococci involved in binding to host cells. Punsalang and Sawyer (1) were unable to inhibit the binding of pili to rabbit erythrocytes with eleven simple sugars. Trust *et al.* (2) were also unable to inhibit attachment of gonococci to buccal cells with ten simple sugars. They did however find evidence to suggest that an oligosaccharide on the surface of the epithelial cell may act as a pilus receptor. Buchanan *et al.* (3) came to a similar conclusion as did Lambden *et al.* (4). King and Swanson (5) were unable to block gonococcal binding to PMN with four simple sugars but found that pretreatment of gonococci with periodate reduced the binding of gonococci to PMN. Watt *et al.* (6) investigated the possibility that gonococcal surface lectins may bind to sugar groups of human fallopian tube cells. They found no inhibition of binding with lectins specific for the following sugars—N-acetyl-galactosamine, galactose, fucose and N-acetyl-glucosamine. They did, however, present evidence suggesting that sugar groups of gonococcal LPS can bind to HE_p-2 cells and concluded that sugars on the gonococcal surface are likely to be involved in attachment to mucosal cells but that appropriate inhibition experiments were required.

Preliminary experiments with gonococci and leukocytes supported the findings of other workers (2, 5) and indicated the absence of simple sugar-inhibitable lectins on gonococci. Efforts were therefore directed at examining sugar-inhibitable lectins on phagocytes that might recognize gonococcal cell wall sugars. Phagocytes have been shown by previous work in the authors' laboratory to express lectins that bind a variety of bacterial species (7).

MATERIALS AND METHODS

Bacteria

N. gonorrhoeae strain E757, recently isolated from a patient attending the Department of Genitourinary Medicine was used throughout these experiments. Opaque colonies of type 2 (T_2), differentiated by means of a Zeiss stereoscopic microscope with a double system of substage lighting, were selectively subcultured and maintained on Difco GC base supplemented as described by Young (8) (GC agar). Suspensions were made from plates with more than 95% of T_2 colonies. Bacteria were harvested from GC agar plates at 14–16 hr, suspended in Dulbecco's phosphate buffered saline (D.PBS) by gentle pipetting, washed and finally re-suspended gently in D.PBS.

Bacterial concentrations were determined at E650 nm on a CE292

Correspondence: Prof. D. M. Weir, Department of Bacteriology, University of Edinburgh Medical School, Teviot Place, Edinburgh EH8 9AG, UK.

0141-2760/84/0300-0107 $03.50

spectrophotometer (CECIL INSTRUMENTS, Cambridge, England) by reference to standards enumerated by microscope counts with a counting chamber.

Phagocytes

Blood from healthy donors with no history of gonorrhoea was collected in plastic tubes containing 10 u of heparin ml^{-1}. Monocytes and PMN were separated on Ficoll-Hypaque cushions at 400 g for 30 min according to the method of Boyum (9). After the interface cells (monocytes and lymphocytes) were collected, the pellet containing granulocytes and red blood cells was re-suspended in 0·82% ammonium chloride to lyse the erythrocytes (10). The separated cells were finally washed twice in D.PBS.

Preparation of Monolayers

PMN and monocytes were re-suspended in Eagle's MEM to give a total cell count of 2×10^5/ml. One millilitre of the above cell preparations was layered onto 13 mm diameter (No. 1) glass coverslips in 16 mm well diameter tissue culture plates (COSTAR, 295 Broadway, Cambridge, Mass.) and incubated for 1 hr at 37°C. Non-adherent cells were removed by washing seven times with D.PBS.

Binding Assay

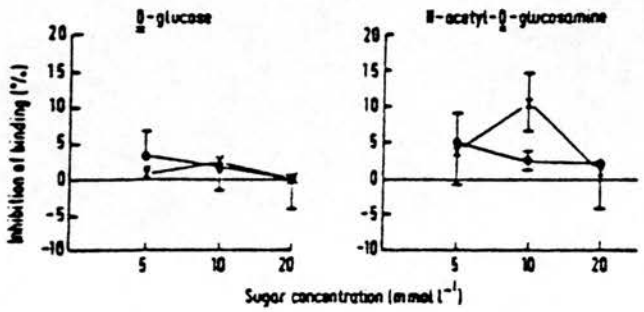
Coverslips with monolayers of PMN or monocytes were overlaid with 1 ml of gonococci in D.PBS containing Ca^{++} and Mg^{++} ions (0·9 mM and 0·5 mM, respectively) and incubated for 2 hr at 4°C. Non-attached organisms were removed by repeated washing with D.PBS. Coverslips were air dried, fixed in methanol and stained with May Grunwald for 3 min, rinsed and stained by Giemsa for 3 min. Bacterial binding was estimated by counting monocytes with bacteria attached at two or more discrete points. Duplicate coverslips were used; for each coverslip, 200 leucocytes were counted and the results expressed as the percentage of cells binding organisms.

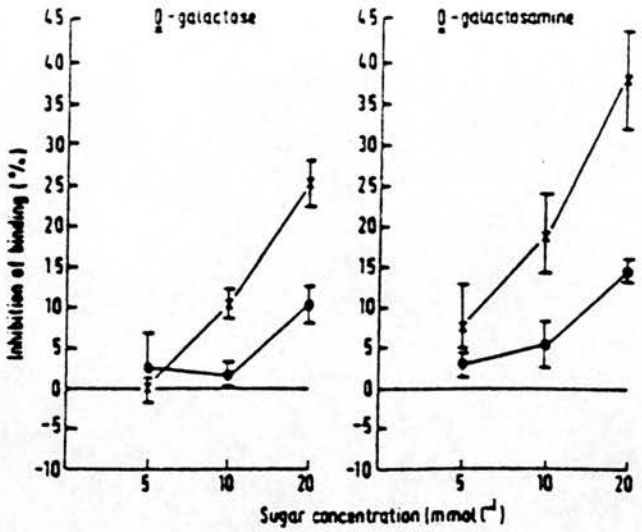
Sugar Inhibition of Gonococcal Binding to Phagocytes

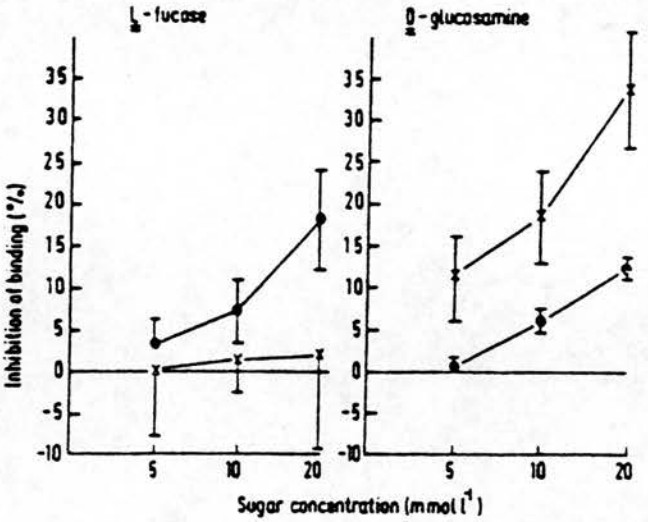
Monocytes and PMN monolayers were prepared as described above. Cell monolayers were preincubated with 0·5 ml of the appropriate sugar solution for 20 min at 4°C. The sugar solutions used were: *D*-galactose, N-acetyl-*D*-galactosamine, *D*-galactosamine, N-acetyl-*D*-glucosamine, *D*-glucose, *L*-fucose and *D*-glucosamine. These sugar solutions were used at 5 mmol l^{-1}, 10 mmol l^{-1} and 20 mmol l^{-1} concentrations in D.PBS with 0·9 mmol l^{-1} Ca^{++} and 0·5 mmol l^{-1} Mg^{++} ions. The cells were then washed and the binding assay performed at 4°C for 2 hr. Pilate gonococci were used at a concentration which gave approximately 50% binding for both monocyte and PMN controls.

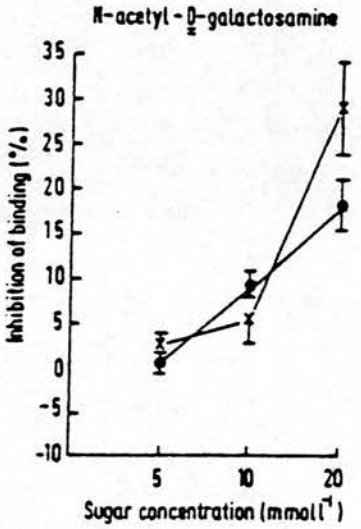
RESULTS

Figures 1 to 4 show the results of sugar inhibition studies on binding of the pilate variant of strain E757 to phagocytes of blood group O. Each figure shows the effect on binding of preincubation of the phagocytes with each









FIGS. 1–4. These figures show the effect of preincubation of phagocytes with the named monosaccharide on the binding of pilate gonococci of strain E757. The assays were carried out with PMN (= X) and monocytes (= ●) of blood group O at 4°C for 2 hr. Each point represents the mean of three experiments ± 1 SEM.

sugar at three different concentrations. Binding of gonococci to PMN and monocytes can be inhibited by *D*-galactose, N-acetyl-*D*-galactosamine, *D*-galactosamine and *D*-glucosamine. Each of these inhibitions follows a dose dependent pattern, with the highest concentration 20 mmol l^{-1} giving the greatest inhibition. N-acetyl-*D*-glucosamine and *D*-glucose had no effect on the binding of gonococci to either PMN or monocytes. *L*-fucose inhibited binding of gonococci to monocytes in a dose dependent fashion but did not inhibit binding of gonococci to PMN. Differences in inhibition for PMN and monocytes were also seen with *D*-glucosamine, with the dose response inhibition of each of these sugars at a lower level for monocytes than PMN.

Discussion

The results reveal that phagocytes are capable of binding gonococci by various sugar-inhibitable "lectin-like" interactions. Each of the four sugars giving clear dose dependent inhibition—*D*-galactose, *D*-galactosamine, N-acetyl-*D*-galactosamine and *D*-glucosamine—are present in the gonococcal outer surface (11, 12, 13).

D-glucose has been detected on the gonococcal outer surface but did not inhibit binding. Work in our laboratory (14) has shown that *D*-glucose did not inhibit binding of *Staphylococcus albus* to human or guinea pig PMN, indicating the absence of a *D*-glucose specific lectin on the phagocytes. In contrast, human monocytes do express a *D*-glucose specific lectin and the failure to inhibit gonococcal attachment to these cells by *D*-glucose suggests that the *D*-glucose present on the gonococcal outer surface was somehow shielded or not exposed exteriorly. There was a notable difference in the ability of *L*-fucose to inhibit binding of gonococci in that inhibition occurred with monocytes but not with PMN. *L*-fucose has the same orientation at C_4 as *D*-glucose and this orientation appears to be important in binding of glycoconjugates to rat alveolar macrophages (15). Thus the differences noted between PMN and monocytes with *L*-fucose may be due to the absence of the *D*-glucose-specific lectin on PMN. Furthermore, quantitative differences are seen with *D*-galactosamine and *D*-glucosamine in their binding to PMN and monocytes. In both cases PMN were more readily inhibited than monocytes, presumably reflecting the numbers of "lectin-like" receptors on the two cell types.

D-glucosamine does inhibit binding of gonococci to human PMN in contrast to the lack of inhibition by *D*-glucose. Thus the amino group on C2 appears to determine recognition. Previous work on the mannosyl, fucosyl, N-acetyl-glucosaminyl receptor on rat alveolar macrophages had also implicated C2 as a determinant of specificity (15). The failure of N-acetyl-*D*-glucosamine to inhibit binding of gonococci to either PMN or monocytes may reflect either the absence of this sugar from the gonococcal cell wall or the inhibition of recognition on adding the acetyl group to *D*-glucosamine. *D*-galactose, *D*-galactosamine and N-acetyl-*D*-galactosamine in contrast are found in the gonococcal cell wall and inhibit binding of gonococci to both PMN and monocytes.

The details of the specificity of phagocyte lectins for different sugars are not understood. Whilst showing preferential specificity for particular sugars, lectins can also interact to a lesser degree with other sugars, e.g., concanavalin A with its main specificity for α-methyl mannoside can also interact with *D*-mannose, *D*-fructose, maltose, isomaltose, trehalose and various lipopolysaccharides and bacterial polysaccharides.

The recent finding in our laboratories that there is an association between the mouse phagocyte lectin that recognizes cell wall sugars of *Staphylococcus albus* and Ia antigens of the major histocompatibility complex (16) further suggests that lectins may show a degree of polygamy in their ability to recognize carbohydrate determinants. Only a small number of genes code for the I-region antigens and, whilst there is extensive polymorphism in a species, polymorphism in an individual is necessarily limited. The I-region antigens of macrophages are thought to be involved in antigen presentation to T-lymphocytes. To carry out this function the I region must be able to recognize a wide range of determinants (17).

Little is known of the role of the human major histocompatibility complex (MHC) in determining susceptibility or resistance to gonorrhoea. A recent study in Singapore indicates that certain HLA haplotypes (A11 and B15) are associated with resistance to gonorrhoea and syphilis amongst Chinese prostitutes. The haplotype HLA-B17 associated with susceptibility is found in 26% of prostitutes with combined infections of syphilis and gonorrhoea compared to 7% in the control population. This haplotype was absent in the prostitutes who were resistant to these infections (18). Compared to the resistant group the susceptible group were found to have poor blastogenic responses in lymphocyte transformation assays to treponemal and gonococcal antigens. So far there is no evidence of any association with the HLA-D region (equivalent to the mouse I region). The importance of the class II MHC antigens in generation of the immune response to bacteria is widely accepted with T-helper cells recognizing bacterial antigens in association with class II antigens on macrophages. The findings in the present study point to the need to explore further the recognition of gonococci by phagocytes and possible association with class II MHC antigens as determinants of susceptibility.

ACKNOWLEDGEMENTS

We are most grateful to Mrs. M. Cole for help in preparation of the manuscript. The work was supported by grants G81/103811/SB and

G979/64/S from the Medical Research Council. Dr. D. F. Kinane is a recipient of the Davidson Research Fellowship, Faculty of Medicine, Edinburgh University.

REFERENCES

1. Punsalang, A. P., Jr. and Sawyer, W. D. (1973). Role of pili in the virulence of *Neisseria gonorrhoeae*. *Infec. Immun.*, **8**, 255–263.
2. Trust, T. J., Lambden, P. R. and Watt, P. J. (1980). The cohesive properties of variants of *Neisseria gonorrhoeae* strain P9: specific pilus-mediated and non-specific interactions. *J. Gen. Microbiol.*, **119**, 179–187.
3. Buchanan, T. M., Chen, K. C. S., Jones, R. B., Hildebrandt, J. F., Pearce, W. A., Hermodsen, M. A., Newland, J. C. and Luchtel, D. L. (1978). Pili and principal outer membrane protein of *Neisseria gonorrhoeae*: immunochemical structural and pathogenic aspects. In *Immunobiology of* Neisseria gonorrhoeae (edited by G. F. Brooks, E. C. Gotschlich, K. K. Holmes, W. D. Sawyer and F. E. Young), pp. 145–154. Am. Soc. Microbiol., Washington D.C.
4. Lambden, P. R., Robertson, J. N. and Watt, P. J. (1980). Biological properties of two distinct pilus types produced by isogenic variants of *Neisseria gonorrhoeae*. *J. Bacteriol.*, **141**, 393–396.
5. King, G. J. and Swanson, J. (1978). Studies on gonococcus infection. XV. Identification of surface proteins of *Neisseria gonorrhoeae* correlated with leukocyte association. *Infec. Immun.*, **21**, 575–584.
6. Watt, P. J., Ward, M. E., Heckels, J. E. and Trust, T. J. (1978). Surface properties of *Neisseria gonorrhoeae*: Attachment to and invasion of mucosal surfaces. In *Immunobiology of* Neisseria gonorrhoeae (edited by G. F. Brooks, E. C. Gotschlich, K. K. Holmes, W. D. Sawyer and F. E. Young), pp. 253–257. Am. Soc. Microbiol., Washington D.C.
7. Weir, D. M. (1980). Surface carbohydrates and lectins in cellular recognition. *Immunol. Today*, **1**, 45–51.
8. Young, H., Patterson, I. C. and McDonald, D. R. (1976). Rapid carbohydrate utilisation test for the identification of *Neisseria gonorrhoeae*. *Br. J. Vener. Dis.*, **52**, 172–175.
9. Bøyum, A. (1968). Isolation of leukocytes from human blood. Further observation. Methyl cellulose dextran and Ficoll as erythrocyte aggregating agents. *J. Clin. Lab. Invest.*, **21** (Suppl. 97), 31–38.
10. Boyle, W. (1968). An extension of the ^{51}Cr release assay for the estimation of mouse cytotoxins. *Transplantation*, **67**, 761–764.
11. Wiseman, G. M. and Caird, J. D. (1977). Composition of the lipopolysaccharide of *Neisseria gonorrhoeae*. *Infec. Immun.*, **16**, 550–556.
12. Perry, M. B., Daoust, V., Diena, B. B., Ashton, F. E. and Wallace, R. (1975). The lipopolysaccharides of *Neisseria gonorrhoeae* colony types 1 and 4. *Can. J. Biochem.*, **53**, 623–629.
13. Stead, A., Main, J. S., Ward, M. E. and Watt, P. J. (1975). Studies on lipopolysaccharides isolated from strains of *Neisseria gonorrhoeae*. *J. Gen. Microbiol.*, **88**, 121–131.
14. Glass, E. J., Stewart, J. and Weir, D. M. (1981). Presence of bacterial binding "lectin-like" receptors on phagocytes. *Immunology*, **44**, 529–534.
15. Shepherd, V. L., Lee, Y. C., Schlesinger, P. L. and Stahl, P. D. (1981). L-fucose terminated glycoconjugates are recognised by pinocytosis receptors on macrophages. *Proc. Natl. Acad. Sci. U.S.A.*, **78**, 1019–1022.
16. Stewart, J., Glass, E. J. and Weir, D. M. (1982). Macrophage binding of *Staphylococcus aureus* is blocked by anti-I region alloantibody. *Nature*, **298**, 852–854.
17. Howie, S. and McBride, W. H. (1982). Cellular interactions in thymus-dependant antibody responses. *Immunol. Today*, **3**, 273–278.
18. Chan, S. H. and Rajan, V. S. (1982). HLA-resistance and susceptibility to venereal disease. In *Immunogenetics in Rheumatology* (edited by R. L. Dawkins, F. T. Christiansen and P. J. Zilko), pp. 20–23. Excerpta Medica, Amsterdam, Oxford, Princeton.

Teviot Scientific Publications Ltd
31 Montpelier Park
Edinburgh EH10 4LX, UK

SHORT COMMUNICATION

Alteration of Pyocin-sensitivity Pattern of *Neisseria gonorrhoeae* is Associated with Induced Resistance to Killing by Human Serum

By F. P. WINSTANLEY,[1] C. C. BLACKWELL,[1] E. L. TAN,[2] P. V. PATEL,[2] N. J. PARSONS,[2] P. M. V. MARTIN[3] AND H. SMITH[2]*

[1] *Department of Bacteriology, University of Edinburgh Medical School, Edinburgh EH8 9AG, UK*
[2] *Department of Microbiology, University of Birmingham, Birmingham B15 2TT, UK*
[3] *Unité d'Ecologie bactérienne, Institut Pasteur, Paris, France*

(*Received 1 February 1984*)

A laboratory-grown strain of *Neisseria gonorrhoeae*, selected *in vivo*, BS4 (agar), is susceptible to complement-mediated killing by fresh human serum but is relatively resistant to killing by human phagocytes. It can be induced to serum resistance by incubation with a small molecular weight fraction of guinea pig serum. The serum-susceptible and induced-resistant forms show differences in pyocin sensitivity tests. This indicates either differences in the structure of their lipopolysaccharides or masking of some determinant(s). The pyocin sensitivity pattern of BS4 (agar) is only slightly different from that of a closely related strain, BSSH, which is more susceptible to killing by human phagocytes.

INTRODUCTION

A strain of *Neisseria gonorrhoeae* selected from a small colony forming, pilate laboratory strain (BS) by three or four passages through plastic chambers implanted subcutaneously in guinea pigs was resistant to killing by human serum and human phagocytes when examined without subculture (Penn *et al.*, 1976, 1977). When cultured in laboratory media, the *in vivo*-selected strain, BS4 (agar), lost its serum resistance but retained its resistance to killing by phagocytes (Penn *et al.*, 1976, 1977). The serum resistance could be restored phenotypically to BS4 (agar) by incubating it for 3 h at 37 °C in a defined medium (DM) containing 0·1% (w/v) bovine serum albumin (BSA) and small molecular weight fractions of either guinea pig or some human sera (Veale *et al.*, 1981; Martin *et al.*, 1981).

The determinant(s) of the induced resistance of BS4 (agar) to killing by human serum has not yet been investigated. The limited supply of host-derived inducer has prevented the production of sufficient organisms for investigation of outer membrane proteins (OMP) and lipopolysaccharides (LPS) comparable to those studies on the culture-stable type of serum resistance shown by strains from disseminated gonorrhoea (Brooks *et al.*, 1978; Lambden *et al.*, 1979; James *et al.*, 1982). An investigation of the LPS of susceptible and induced-resistant forms of BS4 (agar) seems particularly relevant because LPS appears to be the target antigen for the 'natural' antibody responsible for the bactericidal activity of normal human serum (Brooks *et al.*, 1978; Schneider *et al.*, 1982). A method for indicating changes in LPS of *N. gonorrhoeae* became available when it was shown that partially purified, Kageyama R-type pyocins from *Pseudomonas aeruginosa* (Kageyama, 1975), the receptor sites for which reside on the LPS (Meadow & Wells, 1978), reacted with gonococci and could form the basis of a typing system (Morse *et al.*, 1976; Sidberry & Sadoff, 1977; Blackwell *et al.*, 1979). Only small numbers of gonococci are required and the pyocin method has been used to indicate LPS differences in mutational and epidemiological studies on gonococcal sensitivity to serum killing and attachment to polymorphonuclear leucocytes (Guymon *et al.*, 1982; Blackwell *et al.*, 1983;

0022-1287/84/0001-1764 $02.00

Winstanley *et al.*, 1983). This paper reports tests with a range of R-type pyocins on strain BS4 (agar) before and after induction to serum resistance by the low molecular weight fraction from guinea pig serum.

The determinants of the resistance of strain BS4 (agar) to killing by phagocytes have been investigated by comparing it with a closely related phagocyte-susceptible strain, BSSH, selected *in vitro* by colonial appearance from the original parent strain BS (Penn *et al.*, 1977). In this case, sufficient organisms could be obtained for biochemical examination. The putative determinant of resistance was not pili and resided in outer membrane vesicles (OMV) (Parsons *et al.*, 1981). Examination of the proteins of purified OMV from BS4 (agar) and BSSH on sodium dodecyl sulphate polyacrylamide gel electrophoresis associated three OMP with the resistance of BS4 (agar) to killing by phagocytes (Parsons *et al.*, 1982). However, it was thought interesting to include in the pyocin tests a comparison of the phagocyte-resistant [BS4 (agar)] and phagocyte-susceptible (BSSH) strains.

METHODS

Neisseria gonorrhoeae. Strains BS4 (agar) and BSSH were derived, stored, cultured and counted as described previously (Penn *et al.*, 1976, 1977; Parsons *et al.*, 1981, 1982).

Induction of BS4 (agar) to serum resistance. The method used was described by Veale *et al.* (1981): 10^4–10^5 gonococci were incubated for 3 h at 37 °C in equal volumes of DM containing 0·1% (w/v) BSA and an ultrafiltrate of guinea pig serum containing molecules of less than 5000 daltons (Veale *et al.*, 1980; Martin *et al.*, 1981). The serum-susceptible BS4 (agar) used for comparison with the resistant organisms in the pyocin tests (Table 1) had been incubated in DM containing 0·1% BSA for 3 h at 37 °C without the inducer.

Pyocins. Representatives of four of the five groups (R1–R5) of R-type pyocins described by Kageyama (1975) were prepared from the following strains of *P. aeruginosa* and partially purified and stored as described by Blackwell *et al.* (1979). These included: R1 pyocins from strains ISD, 2285, IS6 and IS8; R2 pyocin from strain ISB; R3 pyocin from strain ISE; R5 pyocins from strains ISA and ISC; and two unclassified pyocins from strains R205 and 9579.

Tests for pyocin sensitivity. A suspension (15 µl) of partially purified pyocin was added to a suspension of gonococci (100 µl; 10^4–10^5 c.f.u. ml^{-1} in DM (Veale *et al.*, 1981) in a sterile plastic test tube (10 ml capacity). Control tubes received DM (15 µl) but no pyocin. After 30 min incubation at 37 °C, DM (0·5 ml) was added to each tube. The gonococci were deposited by centrifugation (1500 *g*, 15 min, 37 °C) and the supernatant was removed. The gonococci were resuspended in 0·3 ml DM, which was plated out on haemoglobin agar. After incubation at 37 °C in candle extinction cans the colonies were counted. Results of the experimental samples were recorded as +, ± and − when the viable counts were <5%, <20% (but >5%), and >20%, respectively, of the colony counts for the control tubes. All gonococcal strains were tested against the 10 different pyocins in many tests (see Table 1).

RESULTS

The results are summarized in Table 1. During the 3 h induction period to resistance the serum-susceptible form of BS4 (agar) had lost its sensitivity to the pyocin produced by strain R205 and its partial sensitivity to that produced by strain IS8; also its full sensitivity to the R5 pyocin produced by strain ISC became partial. The serum- and phagocyte-susceptible strain BSSH (Penn *et al.*, 1976, 1977) showed a partial sensitivity to the R1 pyocin from strain IS6 in contrast to the insensitivity of the two forms of BS4 (agar), both of which were relatively resistant to killing by phagocytes (Penn *et al.*, 1976, 1977; N. J. Parsons & H. Smith, unpublished observations). In reactions with the pyocins from strains IS8 and ISC, BSSH behaved similarly to the serum-susceptible form of BS4 (agar). With the pyocin from strain R205, BSSH was less sensitive than the serum-susceptible form of BS4 (agar), whereas the serum-resistant form of BS4 (agar) was fully resistant to this pyocin.

DISCUSSION

The pyocin sensitivity tests suggest that a change in LPS occurs when the serum-susceptible gonococcal strain BS4 (agar) is converted to serum resistance by the low molecular weight inducer from guinea pig serum in the short time of 3 h at 37 °C. Overall, a loss of sensitivity to three pyocins accompanied the increase in serum resistance and this indicates that the LPS

Table 1. *Activity of 10 different pyocins on strain BS4 (agar) before and after induction to serum resistance and on strain BSSH*

Each result was obtained on at least four different tests with each pyocin and at least eight different tests with pyocins from strains IS6, IS8, R205, 9579 and ISC. The differences between the serum-susceptible and serum-resistant forms of BS4 (agar) are bracketed.

Strain of gonococci	Serum resistance	Resistance to phagocyte killing	Pyocin:	Inhibition* by pyocins from the Kageyama group									
				R1				Unclassified		R3	R2	R5	
			Strain:	ISD	2285	IS6	IS8	R205	9579	ISE	ISB	ISA	ISC
BS4 (agar)	−	+		−	−	−	[±]	[+ −]	+	−	−	+	[+ ±]
BS4 (agar)	+	+		−	−	−			+	−	−	+	
BSSH	−	−		−	−	±	±	±	+	−	−	+	+

* Results were recorded as +, ± and − when the viable counts were $<5\%$, $<20\%$ (but $>5\%$), and $>20\%$, respectively, of the counts of the control tubes not containing pyocin.

structure had been altered, since three receptor sites appear to have been removed or modified or masked by a new component. This result complements recent correlations made between the susceptibility of clinical isolates to serum killing and the presence of LPS receptors for the R1 pyocins (Winstanley *et al.*, 1983). Any speculation on the precise changes in LPS structure which accompanied the increase in serum resistance depends on the assumption that observations on pyocin reactions with the LPS of *P. aeruginosa* apply to the LPS of *N. gonorrhoeae*. Mutants of gonococci selected for resistance to R1 pyocins remain sensitive to R5 pyocins, indicating that the receptor for the R5 pyocins is in the portion of the LPS nearest to the cytoplasm and those for R1 pyocins are more distal (Winstanley *et al.*, 1983). These are similar to the results obtained for *P. aeruginosa* (Kageyama, 1975). Little can be drawn from the most marked change in pyocin sensitivity accompanying the increase in serum resistance, namely the complete loss of sensitivity to the unclassified pyocin from strain R205, since its receptor site is unknown even for the LPS of *P. aeruginosa*. It should be noted that in the majority of clinical isolates of gonococci, if the strain is sensitive to R1 pyocins it is also sensitive to the pyocin produced by strain R205 (Blackwell *et al.*, 1979). The smaller losses in sensitivity to the R1 pyocin from strain IS8 and the R5 pyocin from strain ISC suggest that receptor sites both in the polysaccharide side chain and in or near the core of the LPS may have been affected. The receptor sites may not have been produced, or they may have been modified, or even obscured by new structures such as proteins under the influence of the small molecular weight inducing factor whose mechanism of action is unknown. However, one possible masking agency, the formation of a capsule under the influence of the inducer, does not occur; in electron microscopy similar to that which demonstrated capsules on some gonococci (Demarco de Hormaeche *et al.*, 1978), capsules could not be seen on BS4 (agar) organisms either before or after being induced to resistance (P. M. V. Martin, unpublished observations).

It is tempting to suggest that changes in pyocin sensitivity which occur as the result of the action of the small molecular weight inducer indicate changes in LPS which are responsible for the increased resistance to serum killing. However, the evidence at present is only that of association (Smith, 1983) and can only be strengthened by deeper investigations on the connections with serum resistance of the LPS and any other bacterial components (for example, OMP) that may be formed under the influence of the inducer.

REFERENCES

BLACKWELL, C. C., YOUNG, H. & ANDERSON, I. (1979). Sensitivity of *Neisseria gonorrhoeae* to partially purified R-type pyocines and a possible approach to epidemiological typing. *Journal of Medical Microbiology* **12**, 321–335.

BLACKWELL, C. C., KOWOLIK, M., WINSTANLEY, F. P., KINANE, D. F., WEIR, D. N., LAW, J. A. & HO WANG CHOK, Y. L. (1983). ABO blood group and susceptibility to gonoccal infection. I. Factors affecting phagocytosis of *Neisseria gonorrhoeae*. *Journal of Clinical and Laboratory Immunology* **4**, 173–178.

BROOKS, G. F., GOTSCHLICH, E. C., HOLMES, K. K.,

SAWYER, W. D. & YOUNG, F. E. (1978). *Immunology of Neisseria gonorrhoeae*. Washington, DC: American Society for Microbiology.

GUYMON, L. F., ESSER, M. & SHAFFER, W. M. (1982). Pyocin-resistant lipopolysaccharide mutants of *Neisseria gonorrhoeae*: alterations in sensitivity to normal human serum and polymyxin B. *Infection and Immunity* **36**, 541–547.

DEMARCO DE HORMAECHE, R., THORNLEY, M. J. & GLAUERT, A. M. (1978). Demonstration by light and electron microscopy of capsules on gonococci recently grown *in vivo*. *Journal of General Microbiology* **106**, 81–91.

JAMES, J. F., ZURLINDEN, E., LAMMEL, C. J. & BROOKS, G. F. (1982). Relation of protein I and colonial opacity to serum killing of *Neisseria gonorrhoeae*. *Journal of Infectious Diseases* **145**, 37–44.

KAGEYAMA, M. (1975). Bacteriocins and bacteriophages in *Pseudomonas aeruginosa*. In *Microbial Drug Resistance: Report of the International Symposium on Bacterial Resistance*, pp. 291–309. Edited by S. Mitsuhasi & H. Hashimoto. Tokyo: University of Tokyo Press.

LAMBDEN, P. R., HECKELS, J. E., JAMES, L. T. & WATT, P. J. (1979). Variations in surface proteins associated with virulence properties in opacity types of *Neisseria gonorrhoeae*. *Journal of General Microbiology* **114**, 305–312.

MARTIN, P. M. V., PATEL, P. V., PARSONS, N. J. & SMITH, H. (1981). Induction of phenotypically determined resistance of *Neisseria gonorrhoeae* to human serum by factors in human serum. *Journal of General Microbiology* **127**, 213–217.

MEADOW, P. M. & WELLS, P. L. (1978). Receptor sites for R-type pyocins and bacteriophage E79 in the core part of the lipopolysaccharide(s) of *Pseudomonas aeruginosa* PAC1. *Journal of General Microbiology* **108**, 339–343.

MORSE, S. A., VAUGHAN, P., JOHNSON, D. & IGLEWSKI, B. H. (1976). Inhibition of *Neisseria gonorrhoeae* by a bacteriocin from *Pseudomonas aeruginosa*. *Antimicrobial Agents and Chemotherapy* **10**, 354–362.

PARSONS, N. J., KWAASI, A. A. A., TURNER, J. A., VEALE, D. R., PERERA, V. Y., PENN, C. W. & SMITH, H. (1981). Investigation of the determinants of the survival of *Neisseria gonorrhoeae* within human polymorphonuclear phagocytes. *Journal of General Microbiology* **127**, 103–112.

PARSONS, N. J., KWAASI, A. A. A., PERERA, V. Y., PATEL, P. V., MARTIN, P. M. V. & SMITH, H. (1982). Outer membrane proteins of *Neisseria gonorrhoeae* associated with survival within human polymorphonuclear phagocytes. *Journal of General Microbiology* **128**, 3077–3081.

PENN, C. W., SEN, D., VEALE, D. R., WITT, K., PARSONS, N. J. & SMITH, H. (1976). Morphological, biological and antigenic properties of *Neisseria gonorrhoeae* adapted to growth in guinea pig subcutaneous chambers. *Journal of General Microbiology* **97**, 35–43.

PENN, C. W., VEALE, D. R. & SMITH, H. (1977). Selection from gonococci grown *in vitro* of a colony type with some virulence properties of organisms adapted *in vivo*. *Journal of General Microbiology* **100**, 147–158.

SCHNEIDER, H., GRIFFISS, J. M., WILLIAMS, G. D. & PIER, G. B. (1982). Immunological basis of serum resistance of *Neisseria gonorrhoeae*. *Journal of General Microbiology* **128**, 13–20.

SIDBERRY, H. D. & SADOFF, J. C. (1977). Pyocin sensitivity of *Neisseria gonorrhoeae* and its feasibility as an epidemiological tool. *Infection and Immunity* **15**, 628–637.

SMITH, H. (1983). The elusive determinants of bacterial interference with non-specific host defences. *Philosophical Transactions of the Royal Society* **B303**, 99–113.

VEALE, D. R., PENN, C. W., PARSONS, N. J. & SMITH, H. (1980). Preliminary studies of a factor in guinea pig serum which induces serum resistance in *Neisseria gonorrhoeae*. *Society for General Microbiology Quarterly* **7**, 188.

VEALE, D. R., PENN, C. W. & SMITH, H. (1981). Factors affecting the induction of phenotypically determined serum resistance of *Neisseria gonorrhoeae* grown in media containing serum or its diffusible components. *Journal of General Microbiology* **122**, 239–245.

WINSTANLEY, F. P., BLACKWELL, C. C., WEIR, D. M. & KINANE, D. F. (1983). Blood groups and susceptibility to gonococcal infection. II. The relationship of lipopolysaccharide type to gonococcal sensitivity to the bactericidal activity of normal human serum. *Journal of Clinical and Laboratory Immunology* **11**, 27–32.

J. Clin. Lab. Immunol. (1984), 15, 191–194

ABO BLOOD GROUP AND SUSCEPTIBILITY TO URINARY TRACT INFECTION: NO EVIDENCE FOR INVOLVEMENT OF ISOHAEMAGGLUTININS

C. C. BLACKWELL,[1] S. ANDREW,[1] S. J. MAY,[1] D. M. WEIR,[1] C. MACCALLUM,[1] and R. P. BRETTLE[2]
[1]*Department of Bacteriology, The Medical School, University of Edinburgh, Teviot Place, Edinburgh, UK; and* [2]*The City Hospital, Greenbank Drive, Edinburgh, UK*
(Received 19 July 1984)

SUMMARY Investigations of the possible mechanisms underlying the increased susceptibility of women who are blood group B and non-secretors of water-soluble blood group antigen to recurrent urinary tract infection (UTI) revealed that isohaemagglutinins are not involved in bactericidal killing or agglutination of 8 serotypes (1, 2) of *Escherichia coli* frequently associated with UTI. The implications of these results and possible areas for future studies are discussed.

Key words: Blood group, urinary tract infection, isohaemagglutinins

INTRODUCTION

THERE have been a number of reports linking susceptibility to certain infectious diseases with ABO blood group, particularly those involving Gram-negative bacteria such as cholera (3), enteric infections (4, 5) and gonorrhoea (6–8). The antigens of the ABO blood groups are present in two forms: an alcohol soluble form found in tissues and a water soluble form in the body fluids of individuals with the genetically controlled ability to secrete blood group substance (9). Non-secretion of blood group substances has also been linked to susceptibility to certain infections including cholera (10) and rheumatic fever (11).

A survey of women attending a clinic for investigation of recurrent urinary tract infections revealed that those of blood groups B and AB who were also non-secretors showed a significant relative risk of infection of 3·12. This appeared to be a genuine example of synergy in which absence of anti-B isohaemagglutinin and secretor substance combined to give an increased risk of recurrent UTI (12).

Because antigens that cross-react with blood group substances are found on a number of Gram-negative bacteria (7, 13–15), we investigated whether the anti-A and anti-B isohaemagglutinins might contribute to protective humoral immunity to UTI. In the presence of complement, anti-B antibody had been found to be bactericidal for *Escherichia coli* serotype O86 (16). It has also been shown to be an opsonin for serotype O86 (17). Antibodies bactericidal for Gram-negative organisms are often directed toward the lipopolysaccharide (LPS) components (15, 18). These LPS antigens are also the basis for classification of *E. coli* strains into their various serotypes.

We postulated that, as in the case of serogroup 086, the anti-B isohaemagglutinin in subjects of blood groups A and O might be bactericidal for serotypes of *E. coli* associated with UTI, or perhaps they might function as agglutinating or opsonizing antibodies for these strains. The hypothesis is tested in the study reported here.

MATERIALS AND METHODS

Bacteria

Strains of *E. coli* of serogroups O1, O2, O4, O6, O7, O25, O50, and O75 commonly isolated from UTI (1, 2) were obtained from the NATIONAL TYPE CULTURE COLLECTION, PUBLIC HEALTH LABORATORIES, Colindale, London.

They were maintained on nutrient agar (Columbia agar base, Oxoid) and their identity checked by agglutination tests with their respective typing sera (DIFCO LABORATORIES, Detroit, MI). A serum sensitive strain of *Neisseria gonorrhoeae*, P280 (15), maintained on modified New York City medium (MNYC; Young, 1978) was included in all assays for bactericidal activity as a positive control.

Subjects

Control sera were obtained from healthy women attending a postnatal clinic and from women in the Department of Bacteriology. Sera from patients with histories of recurrent UTI were obtained from women attending the outpatient clinic of the City Hospital, Edinburgh. Each sample was heat-inactivated at 56°C for 30 min and stored at −20°C until tested.

Correspondence: Dr. Caroline C. Blackwell, Department of Bacteriology, The Medical School, University of Edinburgh, Teviot Place, Edinburgh EH8 9AG, UK.

0141-2760/84/1200-0191 $03.50

Methods

Micro-bactericidal assays were performed by the method described by Winstanley *et al.* (15). Human AB serum was used as the complement source. It was absorbed for 24 hr at 4°C with a live suspension of each of the *E. coli* serogroups used in the study to remove any cross-reacting antibodies. The absorbed serum was distributed into 200 µl aliquots, and stored at −70°C until needed. The haemolytic titre of the complement source was 32–64.

An 18-hr culture of each strain was suspended in Dulbecco's phosphate-buffered saline supplemented with Mg^{++} and Ca^{++} ions (0·5 mmol and 0·9 mmol respectively) to give approximately 10^4 colony forming units per ml. Serial two-fold dilutions of the sera to be tested were made in microtitre plates and to each was added 40 µl of one of the bacterial suspensions and 10 µl of the human complement source. After incubation at 37°C for 30 min, the numbers of viable organisms in each well were determined by culturing 3 20 µl drops from each on nutrient agar. The bactericidal titre of a serum was the highest dilution causing a reduction in viable count of ⩾ 80% as compared with that of a control to which no complement was added.

Agglutination of the 8 serogroups by serum from women of blood groups A and B was assayed as follows. For each strain, bacterial suspensions were prepared from 5–7 colonies of an overnight growth on nutrient agar in 2 ml of sterile saline and vortexed. For each strain 3 nutrient agar plates were seeded with 0·5 ml of the bacterial suspension and incubated overnight at 37°C. The contents of the plates were harvested in 6 ml sterile saline and this suspension boiled in a glass universal tube for 30 min. The suspensions were stored at 4°C until needed.

Slide agglutination tests were performed by thoroughly mixing 10 µl of serum with 10 µl of one of the bacterial suspensions on a glass microscope slide. The slide was rocked gently for 2 min and the results recorded visually with a good light as follows: − = no agglutination; + = slight agglutination; ++ = clear agglutination; +++ = very strong agglutination.

Heat-inactivated sera from individuals of blood groups A and B were absorbed with suspensions of each of the 8 serotypes associated with UTI and also with O86. The sera were mixed with the bacterial suspensions at 37°C for 30 min. They were then incubated overnight at 4°C with fresh bacteria. The bacteria were removed by centrifugation at approximately 2,000 × g in a Sorvall RT 6000 centrifuge. Control sera were treated in a similar fashion, but an equivalent volume of saline was substituted for the bacterial suspension. Titre of isohaemagglutinins was determined by the addition of the appropriate erythrocyte suspension to serial two-fold dilutions of the absorbed sera and the control sera. The highest dilution at which there was visible agglutination of the red blood cells was noted.

Absorption of blood group substance of *E. coli* was performed by incubating saliva from secretors of blood groups A, B, and O with each individual serotype. Approximately 10^7 cfu/ml of boiled bacteria was incubated with 0·5 ml of saliva for 30 min at 37°C. The suspended bacteria were pelleted at 2,000 × g in a Sorvall RT 6000 centrifuge. The supernates were tested for their ability to inhibit haemagglutination of A, B, and O cells by anti-A, anti-B isohaemagglutinins and the lectin of *Ulex europeus* respectively (20).

Results

Bactericidal Activity of Isohaemagglutinins

The *E. coli* strains of serogroups O1, O2, O4, O6, O7, O25, O50, and O75 were tested in bactericidal assays against a panel of sera from women who had been referred to the UTI clinic at the City Hospital and against a control panel of sera from a population with no recent history of infection. Among the UTI patients there were 13 of blood group A, 32 of group B and 10 of group O. Among the controls there were 6 of group A and 6 of group B. Although each of the sera killed the serum-sensitive gonococcal strain P280, there was no significant bactericidal activity against any of the 8 *E. coli* serogroups.

Table 1 Agglutination of *E. coli* strains by sera from patients with UTI

	% of sera with agglutinating antibodies	
	Blood group	
E. coli *serogroup*	*A* *N = 19*	*B* *N = 20*
O1	84·2	45
O2	31·6	10
O4	89·5	65
O6	21·1	40
O7	63·2	50
O25	89·5	100
O50	57·9	70
O75	57·9	80

Agglutinating Activity of Isohaemagglutinins

Twenty group B sera and 19 group A sera obtained from women attending the UTI clinic were examined for their ability to agglutinate the 8 serogroups associated with infection (table 1). Agglutinating activity was observed for each serogroup. Although there were differences in the reactions observed with A and B sera, e.g. for O1 and O2, these differences were not dependent on isohaemagglutinins. Absorption of serum from A and B individuals with each of the 8 strains did not reduce the titre of either anti-A or anti-B antibodies. Absorption of group A serum with strain O86 removed all the anti-B isohaemagglutinin. In addition, serum samples from 8 controls of blood group AB were found to agglutinate each of the 8 serotypes tested.

Because these 8 serogroups did not absorb isohaemagglutinins, they were not tested for differences in opsonization by autologous serum in phagocytosis experiments with polymorphonuclear leukocytes and monocytes obtained from donors of blood groups A and B.

Absorption of A, B, and H antigens from the saliva of secretors by the 8 serotypes was tested. None of the serotypes was able to remove any of the blood group antigens.

Discussion

Although individuals of blood group B appear to be more susceptible to genitourinary infections by Gram-negative bacteria, the host-parasite interactions underlying these observations remain unclear. In this study we have eliminated any contribution to susceptibility or

resistance to recurrent urinary tract infections by isohaemagglutinins, at least for 8 serotypes commonly associated with these infections.

The target antigen for bactericidal antibodies against Gram-negative bacteria is LPS. Anti-B isohaemagglutinin is bactericidal for serotype O86, but there was no activity directed by anti-A or anti-B antibodies against the LPS structures of the 8 serotypes tested. A number of studies on the bactericidal activity of human serum for the O serotypes of *E. coli* have been undertaken with small numbers of individual sera or pooled sera from a small number of healthy donors. Although there were variations in sensitivities to these sera among different strains of the O serotypes, there was no information regarding the blood group of the individuals tested (2, 21, 22). In each of these studies, serotypes associated with UTI were found to be resistant to the sera tested. Our study confirms these observations for 54 individuals of known blood groups. The K antigens of our strains were not determined because they had been found not to affect serum sensitivity (2).

Since none of the 8 strains absorbed the isohaemagglutinins, these were not the antibodies responsible for the agglutinating activity observed in the sera of the UTI patients. Absorption of the *Ulex* lectin by the *E. coli* strains was not tested because anti-H antibodies are not normally present in humans.

A long-term survey of UTI patients is in progress. We will be examining the serotypes of their *E. coli* isolates to determine if there is a correlation between blood group and serotype; e.g. group B and strains of serotype O2 (table 1).

Since there do not appear to be antigens cross-reacting with blood group A or B antigens on the bacteria tested, an alternative hypothesis was suggested: there are receptors on the bacterial surface that recognize blood group antigens or other antigens containing galactose, fucose, and/or N-acetyl galactosamine on the host's epithelial cells. In this case, water soluble blood group substances in secretions and in urine could block these receptors, reducing the probability of binding by this mechanism.

Our experiments with boiled bacterial cells or cells vortexed to remove fimbriae revealed that the different serotypes did not absorb blood group antigen from the saliva of secretors. This suggests that the LPS does not form part of the hypothetical binding mechanism proposed above. The ability of mannose-sensitive and mannose-resistant fimbriae to absorb blood group antigens and the possible role of these interactions in establishment of infection are being investigated.

These results suggest that the increased susceptibility of women of blood groups B and AB who are non-secretors is not related to the absence of anti-B isohaemagglutinin. These serum-resistant O serotypes most frequently isolated from UTI patients are those most frequently found as part of the normal faecal flora (2). We would agree with these authors that the association of these serogroups with bacteraemia and also with UTI probably reflects a greater opportunity to colonize the urinary tract rather than any particular virulence factor such as an attachment mechanism associated with LPS composition. Our future investigations will concentrate on interaction of the blood group antigens in secretions with protein and glycoprotein components of the bacterial surface.

ACKNOWLEDGEMENTS

We wish to thank Drs. J. McC. Murdoch, J. A. Gray and P. D. Welsby for allowing us to study their patients. We are most grateful to Professor J. G. Collee for his advice and to Mrs. M. Cole for her help with preparation of the manuscript. The work was supported by a grant from the Biomedical Research Committee of the Scottish Home and Health Department (K/MRS/50/C449).

REFERENCES

1. Ørskov, I., Ørskov, F., Jann, B. and Jann, K. (1977). Serology, chemistry and genetics of O and K antigens of *Escherichia coli*. *Bacteriol. Rev.*, **41**, 667.
2. McCabe, W. R., Kaijser, B., Olling, S., Uwaydah, M. and Hanson, L. Å. (1978). *Escherichia coli* in bacteremia: K and O antigens and serum sensitivity of strains from adults and neonates. *J. Infect. Dis.*, **138**, 33.
3. Barua, D. and Paguio, A. D. (1977). ABO blood groups and cholera. *Ann. Hum. Biol.*, **4**, 489.
4. Socha, W., Bilinska, M., Kaczera, Z., Padjak, E. and Stankiewicz, P. (1969). *Escherichia coli* and ABO blood groups. *Folia Biol. (Krakow)*, **17**, 259.
5. Robinson, M. G., Tolchin, D. and Halpern, C. (1971). Enteric bacterial agents and the ABO blood groups. *Am. J. Hum. Genet.*, **23**, 135.
6. Foster, M. T. and Labrum, A. H. (1976). Relation of infection with *Neisseria gonorrhoea* to ABO blood groups. *J. Infect. Dis.*, **133**, 329.
7. Miler, J. J., Novotny, P., Walker, P. D., Harris, J. R. W. and MacLennan, I. P. B. (1977). *Neisseria gonorrhoeae* and ABO isohaemagglutinins. *Inf. Immun.*, **15**, 713.
8. Kinane, D. F., Blackwell, C. C., Winstanley, F. P. and Weir, D. M. (1983). Blood group, secretor status and susceptibility to infection by *Neisseria gonorrhoeae*. *Br. J. Vener. Dis.*, **59**, 44.
9. Race, R. R. and Sanger, R. (1975). *Blood Groups in Man* (6th edn.), p. 547. Blackwell Scientific Publications, Oxford.
10. Chaudhuri, A. and Das Adhikary, C. R. (1978). Possible role of blood-group secretory substances in the aetiology of cholera. *Trans. Roy. Soc. Trop. Med. Hyg.*, **72**, 664.
11. Haverkorn, M. J. and Goslings, W. R. O. (1969). Streptococci, ABO blood groups, and secretor status. *Am. J. Hum. Genet.*, **21**, 360.
12. Kinane, D. F., Blackwell, C. C., Brettle, R. P., Weir, D. M., Winstanley, F. P. and Elton, R. A. (1982). ABO blood group, secretory state and susceptibility to recurrent urinary tract infection in women. *Br. Med. J.*, **285**, 7.
13. Springer, G. F., Williamson, P. and Brandes, W. C. (1961). Blood group activity of Gram-negative bacteria. *J. exp. Med.*, **113**, 1077.
14. Springer, G. F. (1971). Blood group and Forssman antigenic determinants shared between microbes and mammalian cells. *Prog. Allergy*, **15**, 9.
15. Winstanley, F. P., Blackwell, C. C., Weir, D. M. and Kinane, D. F. (1983). ABO blood group and susceptibility of gonococcal infection. II. The relationship of lipopolysaccharide type to gonococcal

sensitivity to the bactericidal activity of normal human serum. *J. Clin. Lab. Immunol.*, **11**, 27.
16. Muschel, L. H. and Osawa, E. (1959). Human blood group substance B in *Escherichia coli* O86. *Proc. Soc. Exp. Biol. New York*, **101**, 614.
17. Check, J. H., O'Neill, E. A., O'Neill, K. E. and Fuscaldo, K. E. (1972). Effect of anti-B antiserum on the phagocytosis of *Escherichia coli*. *Inf. Immun.*, **6**, 95.
18. Schneider, H., Griffiss, J. M., Williams, G. D. and Pier, G. B. (1982). Immunological basis of serum resistance of *Neisseria gonorrhoeae*. *J. Gen. Microbiol.*, **128**, 13.
19. Young, H. (1978). Cultural diagnosis of gonorrhoea with modified New York City (MNYC) medium. *Br. J. Vener. Dis.*, **54**, 36.
20. Mollinson, P. L. (1979). *Blood Transfusion in Clinical Medicine* (6th edn.), p. 414. Blackwell Scientific Publications, Oxford.
21. Vosti, K. L. and Randall, E. (1970). Sensitivity of serologically classified strains of *Escherichia coli* of human origin to the serum bactericidal system. *Am. J. Med. Sci.*, **257**, 114.
22. Olling, S., Hanson, L. Å., Holmgren, J., Jodal, U., Lincoln, K. and Lindberg, U. (1973). The bactericidal effect of normal human serum on *Escherichia coli* strains from normals and from patients with urinary tract infections. *Infection*, **1**, 24.

Teviot-Kimpton Publications
31 Montpelier Park
Edinburgh EH10 4LX, UK

Host-Parasite Interactions Influencing Susceptibility to Diseases Caused by the Pathogenic *Neisseria* Species

C. C. BLACKWELL, F. P. WINSTANLEY, D. M. WEIR, AND D. F. KINANE

Department of Bacteriology, The Medical School, University of Edinburgh, Edinburgh EH8 9AG, Scotland

Evidence from a number of sources suggests that individuals of blood group B are more susceptible than individuals from other blood groups to infections by the pathogenic *Neisseria* species. We have examined humoral and phagocytic immune responses of individuals of the four ABO blood groups to *N. gonorrhoeae* as well as attachment of these bacteria to target epithelial cells of groups A and B. The results provide evidence that might explain, at least in part, some of the host-parasite interactions underlying the epidemiological observations.

Our epidemiological studies have confirmed that there is a significant increase in the proportion of individuals who are blood group B among patients with localized genital gonorrhea (11, 16, 20). There is also indirect evidence to suggest that there may be a similar association for susceptibility to meningococcal disease. The meningitis belt of Africa (24) is found in an area where the incidence of the B gene is highest, 15 to 20% (20).

We have examined three hypotheses that might explain, at least in part, the host-parasite interactions underlying the apparent increased susceptibility of people of blood group B to gonococcal infection. (i) Humoral defenses: As has been reported for *Escherichia coli* O86, anti-B isohemagglutinin of blood groups A and O is bactericidal for certain strains of *Neisseria gonorrhoeae*. (ii) Cellular defenses: As has been reported for *E. coli* O86, anti-B isohemagglutinin is an effective opsonin for certain strains of *N. gonorrhoeae* (8, 22). (iii) Attachment to target epithelial cells: Some strains of gonococci attach in greater numbers to target epithelial cells of group B individuals.

HUMORAL DEFENSES

One of the principal target antigens for bactericidal antibodies is the lipopolysaccharide (LPS) of the goncococcus (12, 27). It is also the receptor for the rod-type (R-type) pyocins of *Pseudomonas aeruginosa* (16). With our pyocin typing system (5) we were able to divide gonococci isolated from both localized genital infections and disseminated gonococcal infections into two categories. For simplicity we refer to group I as those strains with a "simple" LPS, inhibited only by pyocins of the R5 class as defined by Kageyama (14). Strains of group II were those with a "complex" LPS, inhibited by pyocins of the R5 class and also those of class R1. There were, therefore, two different types of LPS target antigen to examine.

"Natural" serum bactericidal activity. Our controls consisted of women attending postnatal clinics, a population with a very low incidence of gonorrhea and high titers of isohemagglutinins.

Although gonococci have been shown to absorb isohemagglutinins (20), we found that natural bactericidal activity of normal human serum directed against isolates from localized genital infections was not associated with either anti-A or anti-B isohemagglutinins. The important factor was the LPS structure: those strains with a complex LPS were serum sensitive and those with a simple LPS were uniformly resistant (32). This supported the finding of Schneider and co-workers (27), who found that serum resistance in gonococci was not due to masking of antigens, blocking antibodies, or deficiencies in complement, but to lack of an LPS determinant.

One implication of these findings is that the serum-sensitive group II organisms would be restricted to localized infections and the group I strains would be found more frequently among isolates from disseminated gonococcal infections. In an earlier study on pyocin types of gonococci from a number of geographic sources, 95 of 110 isolates (86.4%) from localized infections were group II and 15 (13.6%) were group I. In contrast, of the 24 isolates from disseminated gonococcal infections, 10 (41.6%) were group I. Since group II isolates from disseminated gonococcal infections are also serum resistant, this implies that there are at least two different mechanisms of serum resistance (5, 32).

Immune responses to *N. gonorrhoeae*. Experimental immunization of mice with strains of either LPS group I or group II resulted in production of bactericidal antibodies that cross-reacted with strains of the homologous or heterologous LPS type (32). Since normal uninfected individuals have antibodies bactericidal only for group II strains, we examined sera from patients with gonorrhea to determine whether infection resulted in bactericidal activity against group I strains.

Among the 34 sera from infected patients, titers to group II strain P280 ranged from 16 to 280. Very low titers (4 to 8) of antibodies bactericidal for group I strain E757 were detected in sera from 3 patients, but not among the other 31 samples (4).

This lack of production of bactericidal antibodies against group I LPS may result from its stimulation of antibodies that do not activate the classical complement system (immunoglobulin A or G4) or from the failure of the human immune system to respond to that portion of the gonococcal LPS associated with the binding site for R5 pyocins. It has been reported that among Chinese prostitutes susceptibility and resistance to gonorrhea were associated with differences in major histocompatibility complex genes. Resistance was associated with haplotype A11, B15, and susceptibility was associated with B17 (7). Recognition of the group I antigens might depend on the genetic constitution of the individual.

Although we were able to divide nonserogroupable meningococci into the same two groups by the pyocin typing system (2), the same patterns of serum sensitivity and resistance were not found when we tested our control sera for activity against polyagglutinable, autoagglutinable, and nonagglutinable strains. Most of the 48 sera contained bactericidal antibodies for each of the 10 nonserogroupable strains and for strains of serogroups B and W135 but none against a strain of group Y (3; manuscript in preparation).

Sihce the LPS of gonococci and meningococci are structurally similar (2, 28), it was predicted that gonococcal infection might act as a stimulus for bactericidal antibodies against *N. meningitidis*. In contrast to the control population, there were very low titers of these antibodies in the sera of patients with gonorrhea for serogroups A, B, and C. The titers obtained with nonserogroupable strains were similar to the controls (33).

Although the age range at greatest risk of meningococcal disease is 0 to 4 years, the group next most at risk is 15 to 19 years, the age when sexual activity begins and the incidence of gonorrhea is high (15, 25). Outbreaks of meningococcal disease in military establishments occur in the 18- to 24-year age group, also the group with the highest incidence of gonorrhea (4, 30). This suggests that a recent history of gonorrhea may be one of the factors contributing to the increased susceptibility of young adults to diseases caused by *N. meningitidis* (33).

CELLULAR DEFENSES

In our first studies on binding of gonococci by phagocytes (16), it became clear that neutrophils and monocytes interact in very different ways with these bacteria. Gonococci bind to a higher proportion of neutrophils, and the presence or absence of pili does not significantly affect this binding. As with epithelial cells and spermatozoa, a higher proportion of piliated variants of a strain bound to monocytes when compared with the nonpiliated form (13, 29).

There was no significant difference in association of neutrophils with gonococci opsonized by sera containing anti-A or anti-B isohemagglutinins; however, larger numbers of those strains with the complex LPS (group II) than of those strains with the simple LPS (group I) were associated with the neutrophils of each ABO blood group (1).

There were no differences in association of unopsonized gonococci with monocytes from donors of the four ABO groups. When bacteria were opsonized with autologous serum from the donor, there was a significant increase in association with monocytes of groups A, B, and O, but not AB. Group B monocytes bound the greatest number. Absorption of isohemagglutinins from the serum reduced the levels of binding to that observed for unopsonized bacteria (17).

Our original hypothesis was not proved, but if the hypothesis of Novotny and colleagues is correct, that gonorrhea is a "disease of the human macrophage" (23), these results may explain the increased susceptibility of group B individuals to infection. If gonococci ingested by mononuclear phagocytes are able to inhibit the bactericidal activities of the cell and grow within it, when the cell eventually lyses, the bacteria are released as "infectious units," protected by a coat of debris. The findings of Witt et al. (34) and Veale et al. (31) indicated that gonococci are capable of survival and growth within both neutrophils and monocytes.

The increased association of gonococci with B monocytes may result in an increased probability of forming the infectious units in which the bacteria are shielded from both cellular and humoral immune mechanisms.

ATTACHMENT TO TARGET EPITHELIAL CELLS

Attachment of gonococci to susceptible epithelial surfaces is the first step in the initiation of disease. In these experiments we examined the adherence of two strains from disseminated gonococcal disease to uroepithelial cells from women of blood groups A and B to test our hypothesis. The two strains were obtained from Joan S. Knapp, Neisseria Ref-

erence Laboratory, U.S. Public Health Service, Seattle, Wash. Strain 6354 was LPS group I, prototrophic, serotype WI; strain 7423 was LPS group II, prototrophic, serogroup WI. For both, the MIC of penicillin was 0.125 U/ml.

The bacteria were grown on chocolate blood agar in an atmosphere of 5 to 10% CO_2, harvested at 12 to 13 h in Dulbecco phosphate-buffered saline containing Ca^{2+} and Mg^{2+} (0.9 mM and 0.5 mM, respectively), and adjusted to 5×10^8 cells per ml.

Uroepithelial cells were collected at midmorning from women (ages 22 to 26 years) of blood groups A and B, washed three times in saline, and adjusted to 2×10^6 cells per ml in Dulbecco phosphate-buffered saline.

Duplicate samples of equal volumes of cells and bacteria were gently mixed and incubated for 1 h at 37°C and 50 rpm in an orbital incubator. The mixtures were washed three times with saline and resuspended to the original volume of the reaction mixture. Slides of the washed mixture were prepared from 0.1-ml volumes with a cytospin (Shandon). The slides were fixed with methanol, Gram stained, and examined by light microscopy (Leitz Laborlux). For each slide 40 epithelial cells with adherent bacteria were counted; those cells that could not be counted accurately, those in clumps or that had a distorted morphology, were not included. When there were more than 50 bacteria per cell, the number was recorded as 50.

The experiments were performed once a week over a 4-month period to determine any effects of the menstrual cycle on attachment.

The results in Fig. 1 indicate that more bacteria of both strains attached to the blood group B cells than to the group A cells. There were two to four experiments for each quarter of the cycle except for the first, for which there was only one set of data.

These results agree with those found for attachment of gonococci to vaginal epithelial cells at different stages of the menstrual cycle. More gonococci were found to attach to cells obtained during the postmenstrual phase than to those obtained during the premenstrual phase (10). In a later study Forslin and Danielsson reported no variation in adherence of gonococci to uroepithelial cells during the menstrual cycle (9), but their methods were different from ours in that our subjects were followed over approximately four cycles. The differences in attachment to cells of different blood groups may, in conjunction with the hormonal effects reported by Forslin and co-workers (10), account for the variations observed among different donors by other investigators (29).

These are preliminary experiments that must be repeated with more individuals and also with strains from localized infections. If these observations can be substantiated, this may be another of the factors underlying the increased incidence of group B individuals among gonorrhea patients.

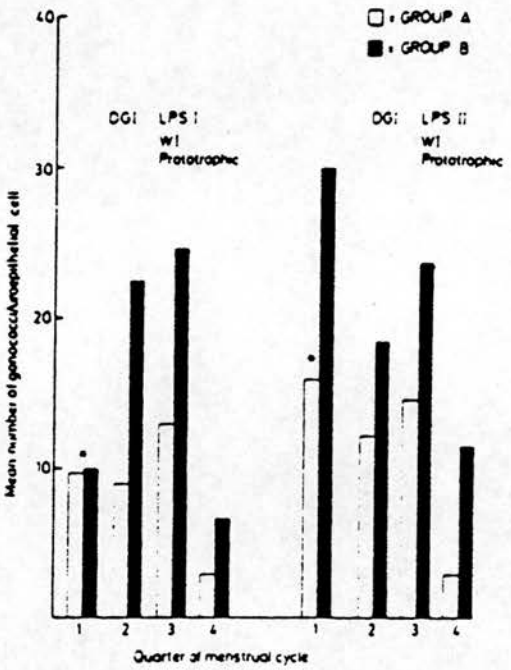


FIG. 1. Attachment of two strains from disseminated gonococcal infections (DGI) to uroepithelial cells obtained from women of blood groups A and B.

There are lectinlike receptors on human phagocytic cells that bind gonococci by means of sugars in their cell envelope (19). We are investigating the suggestion that differences in attachment to target cells of the ABO blood groups may be due to recognition of an adhesin(s) on the bacteria by a similar mechanism.

ACKNOWLEDGMENTS

We are most grateful to M. Cole for help in preparation of the manuscript and to Katherine Reid for serotyping the strains from disseminated gonococcal infection.

The work was supported by grants G81/103811/SB and G979/64/S from the Medical Research Council. D.F.K. was a recipient of the Davidson Research Fellowship, Faculty of Medicine, Edinburgh University.

LITERATURE CITED

1. Blackwell, C. C., M. J. Kowolik, F. P. Winstanley, D. F. Kinane, D. M. Weir, J. A. Law, and Y. L. Ho Wang Chok. 1983. ABO blood group and susceptibility to gonococcal infection. I. Factors affecting phagocytosis of *Neisseria gonorrhoeae*. J. Clin. Lab. Immunol. 10:173–178.
2. Blackwell, C. C., and J. A. Law. 1981. Typing of non-serogroupable *Neisseria meningitidis* by means of sensitivity to R-type pyocins of *Pseudomonas aeruginosa*. J. Infect. 3:370–378.
3. Blackwell, C. C., F. P. Winstanley, and D. M. Weir. 1984. Lipopolysaccharide type and sensitivity of the

pathogenic *Neisseria* to bactericidal activity of normal human serum. Med. Trop. (Marseille) 43:196.
4. Blackwell, C. C., F. P. Winstanley, D. M. Weir, and D. F. Kinane. 1984. Absence of bactericidal antibodies against group I lipopolysaccharide determinants of *Neisseria gonorrhoeae* during human infection. J. Med. Microbiol. 17:353–356.
5. Blackwell, C. C., H. Young, and I. Anderson. 1979. Sensitivity of *Neisseria gonorrhoeae* to partially purified R-type pyocines and a possible approach to epidemiological typing. J. Med. Microbiol. 12:321–335.
6. Bristow, W. M., P. F. D. van Peenen, and R. Volk. 1965. Epidemic meningitis in naval recruits. Am. J. Public Health 55:1039–1045.
7. Chan, S. H., and V. S. Rajan. 1982. HLA—resistance and susceptibility to venereal disease, p. 20. *In* R. L. Dawkins, F. T. Christiansen, and P. J. Zilko (ed.), Immunogenetics in rheumatology. Excerpta Medica, International Congress Series no. 602, Amsterdam.
8. Check, J. H., E. A. O'Neill, K. E. O'Neill, and K. E. Fuscaldo. 1972. Effect of anti-B antiserum on the phagocytosis of *Escherichia coli*. Infect. Immun. 6:95–96.
9. Forslin, L., and D. Danielsson. 1980. *In vitro* studies of the adherence of *Neisseria gonorrhoeae* and other urogenital bacteria to vaginal and uroepithelial cells, with special regard to the menstrual cycle. Gynecol. Obstet. Invest. 11:327–340.
10. Forslin, L., D. Danielsson, and V. Falk. 1979. Variations in attachment of *Neisseria gonorrhoeae* to vaginal epithelial cells during the menstrual cycle and early pregnancy. Med. Microbiol. Immunol. 167:231–238.
11. Foster, M. T., and A. H. Labrum. 1976. Relation of infection with *Neisseria gonorrhoeae* to ABO blood groups. J. Infect. Dis. 133:329–330.
12. Glynn, A. A., and M. E. Ward. 1970. Nature and heterogeneity of the antigens of *Neisseria gonorrhoeae* involved in the serum bactericidal reaction. Infect. Immun. 2:162–168.
13. James-Holmquest, A. N., J. Swanson, T. M. Buchanan, R. D. Wende, and R. P. Williams. 1974. Differential attachment by pilated and non-pilated *Neisseria gonorrhoeae* to human sperm. Infect. Immun. 9:897–902.
14. Kageyama, M. 1975. Bacteriocins and bacteriophages in *Pseudomonas aeruginosa*, p. 291–305. *In* S. Mitsuhashi and H. Hashimoto (ed.), Microbial drug resistance. University Park Press, Baltimore.
15. Kallings, L. O., and I. Moberg. 1977. Epidemiology of gonorrhoea, p. 2–15. *In* F. A. Skinner, P. D. Walker, and H. Smith (ed.), Gonorrhoea, epidemiology and pathogenesis. FEMS Symp. no. 2. Academic Press, London.
16. Kinane, D. F., C. C. Blackwell, D. M. Weir, and F. P. Winstanley. 1982. Phagocyte recognition of *Neisseria gonorrhoeae*. J. Clin. Lab. Immunol. 9:169–172.
17. Kinane, D. F., C. C. Blackwell, D. M. Weir, and F. P. Winstanley. 1983. ABO blood groups and susceptibility to gonococcal infection. III. Role of isohaemagglutinins in increased association of *Neisseria gonorrhoeae* to monocytes from blood group B individuals. J. Clin. Lab. Immunol. 12:83–86.
18. Kinane, D. F., C. C. Blackwell, F. P. Winstanley, and D. M. Weir. 1983. Blood group, secretor status and susceptibility to infection by *Neisseria gonorrhoeae*. Br. J. Vener. Dis. 59:44–46.
19. Kinane, D. F., D. M. Weir, C. C. Blackwell, and F. P. Winstanley. 1984. Binding of *Neisseria gonorrhoeae* by lectin-like receptors on human phagocytes. J. Clin. Lab. Immunol. 13:107–110.
20. Miler, J. J., P. Novotny, P. D. Walker, J. R. W. Harris, and I. P. B. MacLennan. 1977. *Neisseria gonorrhoeae* and ABO isohemagglutinins. Infect. Immun. 15:713–719.
21. Mourant, A. E. 1983. Blood relations. Blood groups and anthropology, p. 134. Oxford University Press, Oxford.
22. Muschel, L. H., and E. Osawa. 1959. Human blood group substance B in *Escherichia coli* O86. Proc. Soc. Exp. Biol. 101:614–617.
23. Novotny, P., J. A. Short, M. Hughes, J. J. Miler, C. Syrett, W. H. Turner, J. R. W. Harris, and I. P. B. MacLennan. 1977. Studies on the mechanisms of pathogenicity of *Neisseria gonorrhoeae*. J. Med. Microbiol. 10:346–365.
24. Peltola, H. 1983. Meningococcal disease: still with us. Rev. Infect. Dis. 5:77–91.
25. Peltola, H., J. M. Kataja, and P. H. Makela. 1982. Shift in the age distribution of meningococcal disease as predictor of an epidemic? Lancet ii:595–597.
26. Sadoff, J. C., W. D. Zollinger, and H. Sidberry. 1978. Cell surface structures of *Neisseria gonorrhoeae*, p. 83–100. *In* G. F. Brooks, E. C. Gotschlich, K. K. Holmes, W. D. Sawyer, and F. E. Young (ed.), Immunobiology of *Neisseria gonorrhoeae*. American Society for Microbiology, Washington, D.C.
27. Schneider, H., J. M. Griffis, G. D. Williams, and G. B. Pier. 1982. Immunological basis of serum resistance of *Neisseria gonorrhoeae*. J. Gen. Microbiol. 128:13–22.
28. Tramont, E. C., J. C. Sadoff, and M. S. Artenstein. 1974. Cross reactivity of *Neisseria gonorrhoeae* and *Neisseria meningitidis* and the nature of the antigens involved in the bactericidal reaction. J. Infect. Dis. 130:240–247.
29. Tramont, E. C., and C. Wilson. 1977. Variations in buccal cell adhesion of *Neisseria gonorrhoeae*. Infect. Immun. 16:709–711.
30. Varela, G. E., and G. D. Gilmore. 1971. Meningococcal disease outbreak at Fort Leonard Wood, Missouri. Mil. Med. 136:723–727.
31. Veale, D. R., M. Goldner, C. W. Penn, J. Ward, and H. Smith. 1979. The intracellular survival and growth of gonococci in human phagocytes. J. Gen. Microbiol. 113:383–393.
32. Winstanley, F. P., C. C. Blackwell, D. M. Weir, and D. F. Kinane. 1983. ABO blood groups and susceptibility to gonococcal infection. II. The relationship of lipopolysaccharide type to gonococcal sensitivity to the bactericidal activity of normal human serum. J. Clin. Lab. Immunol. 11:27–32.
33. Winstanley, F. P., C. C. Blackwell, D. M. Weir, and D. F. Kinane. 1983. Gonorrhoea: a predisposing factor for meningococcal disease? Lancet ii:1134–1135.
34. Witt, K., D. R. Veale, and H. Smith. 1976. Resistance of *Neisseria gonorrhoeae* to ingestion and digestion by phagocytes of human buffy coat. J. Med. Microbiol. 9:1–12.

Dossier : Infections

Biomedicine & Pharmacotherapy, 1985, 39, 167-170.

FACTORS INFLUENCING HOST SUSCEPTIBILITY TO MENINGOCOCCAL DISEASE

by F. P. Winstanley, C. C. Blackwell and D. M. Weir

(Department of Bacteriology, The Medical School, University of Edinburgh*, Edinburgh)

ABSTRACT

Host-parasite interactions influencing the development of the protective humoral immune response to Neisseria meningitidis *are briefly reviewed. Possible consequences of the observed decreased titres of bactericidal activity specific for meningococcal serogroups A, B and C among patients with gonorrhoea are discussed with reference to: 1) the epidemiology of the two diseases, 2) the protective role of « natural » antibodies to the* Neisseria *species and 3) the carriage rate of serogroupable strains of* N. meningitidis *among patients with gonorrhoea and a control population.*

ABRÉGÉ

Les interactions hôte-parasite influençant le développement des réponses immunes humorales protectrices vis-à-vis de Neisseria meningitidis *sont passées en revue. Les conséquences éventuelles des titres bas d'activité bactéricide spécifique des sérogroupes de méningocoque A, B et C observés chez les patients atteints de gonorrhée sont discutées en fonction : 1) de l'épidémiologie des deux maladies ; 2) du rôle protecteur des anticorps « naturels » vis-à-vis des souches de* Neisseria ; *3) de la fréquence de l'état porteur de souches de* N. meningitidis *sérogroupables chez les patients atteints de gonorrhée et dans la population témoin.*

Current knowledge of the factors that determine the susceptibility of individuals to meningococcal disease is scanty, but a number of predisposing conditions have been suggested. These factors include overcrowding, poor general health and living conditions, influenza and absence of serum bactericidal activity against the meningococcus (24). The study of Goldschneider and colleagues (9) showed that immunity to meningococcal disease is related to the titre of serum bactericidal antibody reactive with polysaccharide antigens of *Neisseria meningitidis*.

Their evidence was as follows:

1) The incidence of meningococcal disease is age-dependent. Great susceptibility in the early years of life is followed by an increase in resistance, directly related to the increase in titre of serum antibodies bactericidal for *N. meningitidis*.

2) Low titres or absence of these bactericidal antibodies were shown to correlate with susceptibility of army recruits to meningococcal disease.

3) Passive immunisation with group-specific anti-meningococcal serum had been shown to reduce the case fatality rate in meningococcal disease (7).

These bactericidal antibodies are generally absent or of low titre during the first two years of life, the period of greatest susceptibility to the disease. As the titres of serogroup-specific antibodies increase in older children, the incidence of meningococcal disease decreases. The antigenic stimuli responsible for these antibodies are thought to include contributions from the normal bacterial flora of the body. Carriage of *Neisseria lactamica*, a bacterium closely related to the meningococcus, can provoke the development of cross-reacting bactericidal antibodies to serogroups A, B and C. Gold and co-workers (8) suggested that since this organism is acquired by most infants during the first two years of life, it may be a major stimulant of bactericidal antibodies that cross-react with *N. meningitidis*. Asymptomatic carriage of serogroupable and non-serogroupable meningococci has also been shown to stimulate production of bactericidal antibodies (10, 26). Cross-reactions between meningococci of groups A and C and enteric bacteria have also been observed (27). This evidence suggests that natural immunity to meningococcal disease may be attributable to fortuitous cross-reactions of antibodies stimulated by both generically related and unrelated bacteria.

Artenstein and Ellis (3) pointed out that the high rate of carriage of meningococci compared with the rarity of meningococcal disease suggests that deficiencies in host defence may play a major role in determining systemic invasion. For example, recurrent meningococcal meningitis (18) and gonococcaemia (25) occur frequently in individuals with congenital absence of the terminal components of the complement system. Another genetic factor associated with susceptibi-

* Teviot Place, Edinburgh EH8 9AG, U.K.

Reprints: C. C. Blackwell.

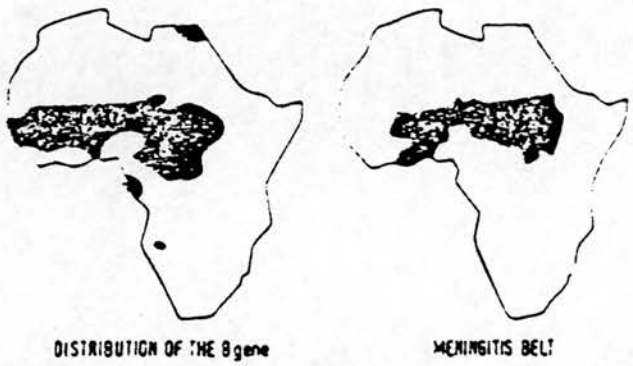


FIG. 1.
Comparison of the meningitis belt of Africa with areas of the continent in which there is a high incidence (15-20 %) of the blood group B gene in the population.

lity to infectious diseases is ABO blood group (21). Individuals of blood group B appear to be more susceptible to infection by *Neisseria gonorrhoeae* than individuals of other ABO blood groups (17); they might also be at greater risk of infection with meningococci.

The congruity between the « meningitis belt » of Africa and the large region of that continent that has a high incidence (15-20 %) of the B gene has not escaped our notice (Figure) (24, 20). A combination of environmental and genetic factors present in this region might be responsible for the high prevalence of meningococcal disease.

In the course of studies on serum-sensitivity among the pathogenic *Neisseria* species, we found that in individuals with active gonorrhoea the levels of antibodies bactericidal for serogroups A, B and C of *N. meningitidis* were much lower than those from an uninfected control population. The markedly reduced titres of bactericidal activity were restricted to the three serogroupable strains tested; the titres against two non-serogroupable strains (0452 and NG8461 were similar to those from « normal » individuals from the same geographical area (31). We suggested that the absence or low titre of bactericidal antibody reactive with serogroups A, B and C in patients with gonorrhoea might result in their resistance to meningococcal disease being compromised.

The following epidemiological observations also suggest a link between susceptibility to meningococcal disease and to gonorrhoea:

a) During an outbreak of meningococcal disease the age range in which greatest morbidity occurs is 0-4 years. These are children who have not yet developed bactericidal antibodies against meningococci (10). The group in whicht he next highest incidence occurs is the 15-19 year olds, an age range corresponding to the onset of sexual activity and in which there is a high incidence of gonorrhoea (16, 23).

b) Outbreaks of meningococcal disease in military institutes occur predominantly among recruits, 80 % of infections developing within 5-6 weeks of their entry (4, 29). The majority of patients with meningococcal disease in an outbreak in a military establishment fell into the age range of 18-24 years which is identical to that in which most military cases of gonorrhoea are to be found (18, 30).

c) The incidence of meningococcal colonisation of the pharynx is higher in patients with genital gonorrhoea (approximately 26 %) than in those without (approximately 11 %). The relative risk of harbouring meningococci when infected with gonococci is 2.44 (95 % confidence limits, 1.4-4.2) (22, 32).

These observations suggest that gonococcal infection or exposure to infection may be one of the factors associated with the increased incidence of meningococcal disease among young adults.

Three hypotheses have to be considered in evaluating this information: (1) a deficiency in bacterial antibody predisposes individuals to infection by both *N. meningitidis* and *N. gonorrhoeae*; (2) IgA antibodies stimulated by gonococcal infection cross-react with determinants on serogroupable meningococci to which the natural bactericidal antibodies are directed, so inhibiting bacteriolysis; (3) infection with *N. gonorrhoeae* may reduce titres of protective cross-reactive antibodies, and in doing so, compromise host defences

to *N. meningitidis*. In contrast to the congenital complement deficiencies, hypotheses 2 and 3 suggest a transient, acquired susceptibility to infection.

There is evidence from two sources to cast doubt on the first hypothesis. In our studies, the patients with gonorrhoea had normal titres of antibody against non-serogroupable meningococci (31) and against serum-sensitive strains of gonococci (3). It is generally accepted that bactericidal antibodies play an important role in the prevention of meningococcal disease, but the role of these antibodies in prevention of localized gonorrhoea is still debatable.

Griffiss has suggested that blocking IgA antibodies to meningococcal antigens may play a role in the pathogenesis of the disease (12). If, as in the case of *N. gonorrhoeae*, bactericidal activity is due to « natural antibodies » of the IgM class (28), then low titres of IgA blocking antibodies in patients sera would produce a prozone (13). This was not observed in our experiments; however, Kahty and colleagues (15) observed the development of meningococcal disease in the presence of high levels of serum antibody to the meningococcal polysaccharide antigens. In their study, over a quarter of the antibodies (28.3 %) were of the IgA class. The phenomenon appeared to occur in conjunction with asymptomatic carriage of *N. meningitidis* or closely related bacteria; this is also found during ano-genital gonococcal infection (13).

The third hypothesis suggests that anti-meningococcal antibodies may be absorbed by cross-reacting antigens during gonococcal infections. Antigenic similarities between gonococcal and meningococcal lipopolysaccharides (LPS) have been observed (1) and *Neisseria* species are known to release free LPS and outer membrane vesicles during growth (5, 14). These may act as absorbents of antibody, possibly leading to the observed reduction (transient?) in antibodies bactericidal for the serogroupable strains tested. A similar phenomenon occurs during pneumococcal infection (6).

The presence of bactericidal antibody has been shown to reduce nasopharyngeal carriage of *N. meningitidis*, and this reduction appeared to be group-specific (11). Young and colleagues observed that women with gonorrhoea were more likely to be colonised by serogroupable meningococci than were women without gonorrhoea. There were no significant differences in colonisation of the two groups by non-serogroupable strains of the organism (33). These findings are additional albeit, indirect evidence for our suggestion that recent gonococcal infection may be one of the many factors contributing to susceptibility to meningococcal disease and could partially account for the higher incidence of this infection among young adults.

ACKNOWLEDGMENTS

We are most grateful to Mrs. M. Cole for help in preparation of the manuscript and to Professor J. G. Collee for his helpful criticism. The work was supported by grants G81/103811 SB and G797/64/S from the Medical Research Council.

REFERENCES

1. Apicella M. A., Bennett K. M., Hermerath C. A. & Roberts E. D. Monoclonal antibody analysis of lipopolysaccharide from *Neisseria gonorrhoeae* and *Neisseria meningitidis*. *Infect. Immun.*, 1981, *34*, 751.
2. Artenstein M. S. & Ellis R. E. The risk of exposure to a patient with meningococcal meningitis. *Milit. Med.*, 1968, *133*, 474.
3. Blackwell C. C., Winstanley F. P., Weir D. M. & Kinane D. F. Absence of bacterial antibodies against group I lipopolysaccharide determinants of *Neisseria gonorrhoeae* during human infection. *J. med. Microbiol.*, 1984 (in press).
4. Bristow W. M., Van Peenen P. F. D. & York R. Epidemic meningitis in naval recruits. *Am. J. Pub. Health*, 1965, *55*, 1039.
5. DeVoe I. W. & Gilchrist J. E. Release of endotoxin in the form of cell wall blebs during in vitro growth of *Neisseria meningitidis*. *J. exp. Med.*, 1973, *138*, 1156.
6. Downie A. W. The specific capsular polysaccharide of pneumococcus Type I and the immunity which it produces in mice. *J. Path.*, 1937, *45*, 131.
7. Flexner S. The results of the serum treatment in 1,300 cases of epidemic meningitis. *J. exp. Med.*, 1913, *17*, 553.
8. Gold R., Goldschneider I., Lepow M. L., Draper T. F. & Randolf M. Carriage of *Neisseria meningitidis* and *Neisseria lactamica* in infants and children. *J. infect. Dis.*, 1978, *137*, 112.
9. Goldschneider I., Gotschlich E. C. & Artenstein M. S. Human immunity to the meningococcus. I. The role of humoral antibodies. *J. exp. Med.*, 1969, *129*, 1307.
10. Goldschneider I., Gotschlich E. C. & Artenstein M. S. Human immunity to the meningococcus. II. Development of natural immunity. *J. exp. Med.*, 1969, *129*, 1327.
11. Gotschlich E. C., Goldschneider I. & Artenstein M. S. Human immunity to the meningococcus. V. The effect of immunisation with meningococcal group C polysaccharide on the carrier state. *J. exp. Med.*, 1969, *129*, 1367.
12. Griffiss J. M. Bactericidal activity of meningococcal antisera blocking by IgA of lytic antibody in human convalescent sera. *J. Immunol.*, 1975, *114*, 1779.
13. Griffiss J. M. & Goroff D. K. IgA blocks IgM and IgG-initiated immune lysis by separate molecular mechanisms. *J. Immunol.*, 1983, *130*, 2882.
14. Johnson K. G., Perry M. B., McDonald I. J. & Russell R. R. B. Cellular and free lipopolysaccharides of some species of *Neisseria*. *Can. J. Microbiol.*, 1975, *21*, 1969.
15. Kahty I., Jonsmes-Somer H., Peltola H. & Makela P. H. Antibody response to capsular polysaccharides of Groups A and C *Neisseria meningitidis* and *Haemophilus influenzae* type B during bacteremic disease. *J. infect. Dis.*, 1981, *143*, 32.
16. Kallings L. O. & Moberg I. Epidemiology of gonorrhoeae. *In* Gonorrhoea. Epidemiology and Pathogenesis. FEMS Symposium No. 2. Skinner F. A., Walker P. D. & Smith H., eds., *Academic Press*, London, 1977.
17. Kinane D. F., Blackwell C. C., Winstanley F. P. & Weir D. M. Blood group, secretor status and susceptibility to infection by *Neisseria gonorrhoeae*. *Brit. J. vener. Dis.*, 1983, *59*, 44.
18. Koprex G. M., Ellenbogen C. & Gerrard R. J. Group Y meningococcal disease in United States Air Force recruits. *Am. J. Med.*, 1977, *62*, 561.
19. Lim D., Gewurz A., Lint T. F., Ghaze M., Sepheri B. & Gewurz H. Absence of the sixth component of complement in a patient with repeated episodes of meningococcal meningitis. *J. Pediat.*, 1976, *89*, 42.
20. Mourant A. E. Blood Relations. Blood groups and anthropology. *Oxford University Press*, Oxford, 1983.
21. Mourant A. E., Kopec A. C. & Domaniewska-Sobczak K. Blood groups and diseases. *Oxford University Press*, Oxford, 1978.
22. Odegaard K. & Gedde-Dahl T. W. Frequency of simultaneous carriage of *Neisseria gonorrhoeae* and *Neisseria meningitidis*. *Brit. J. vener. Dis.*, 1979, *55*, 334.
23. Peltola H., Kataja J. M. & Makela P. H. Shift in the age distribution of meningococcal disease as predictor of an epidemic? *Lancet*, 1982, *ii*, 595.

24. Pettola H. Meningococcal diseases. Still with us. *Rev. infect. Dis.* 1983, *5*, 71.
25. Petersen B. H., Graham J. A. & Brooks G. F. Human deficiency of the eighth component of complement: the requirement for C8 for serum *Neisseria gonorrhoeae* bactericidal activity. *J. clin. Invest.* 1976, *57*, 283.
26. Reller L. B., McGregor R. R. & Beatty H. N. Bactericidal antibody after colonization with *Neisseria meningitidis*. *J. infect. Dis.* 1973, *127*, 56.
27. Robbins J. B., Myerowitz R. L., Whisnant J. K., Argaman M., Schneerson R., Handzel Z. T. & Gotschlich E. C. Enteric bacteria cross-reactive with *Neisseria meningitidis* groups A and C and *Diplococcus pneumoniae* Types I and III. *Infect. Immun.* 1972, *6*, 651.
28. Schoolnik G. K., Ochs H. D. & Buchanan T. M. Immunoglobulin class responsible for gonococcal bactericidal activity of normal human sera. *J. Immunol.* 1979, *122*, 1771.
29. Vareta G. E. & Gilmore G. D. Meningococcal disease outbreak at Fort Leonard Wood, Missouri. *Milit. Med.*, 1971, *136*, 723.
30. White P. C. & Blount J. H. Venereal disease control in the 2nd Marine Division, Camp Lejeune, North Carolina. *Milit. Med.*, 1967, *132*, 252.
31. Winstanley F. P., Blackwell C. C., Weir D. M. & Kinane D. F. Gonorrhoea, a predisposing factor to meningococcal disease. *Lancet*, 1983, *ii*, 1134.
32. Young H., Harris A. B. & Robertson D. H. H. Individual susceptibility to neisserial infection. *Brit. J. vener. Dis.*, 1979, *55*, 188.
33. Young H., Harris A. B., Robertson D. H. H. & Fallon R. J. Anogenital gonorrhoea and pharyngeal colonisation with meningococci: a serogroup analysis. *J. infect. Dis.*, 1983, *9*, 49.

Biomedicine & Pharmacotherapy, 1985, *39*, 170-173.

INFECTION AND *BRANHAMELLA CATARRHALIS*

by D. T. McLeod*, F. Ahmad** and M. A. Calder**

(Chest Unit * and Department of Bacteriology **, City Hospital, Edinburgh)

ABSTRACT

Of the Gram-negative cocci found in the nasopharynx to which any pathogenic status can be attributed, Neisseria meningitidis *and* Neisseria (Branhamella) catarrhalis *have gained significant notoriety. Traditionally,* B. catarrhalis *is regarded as a nasopharyngeal commensal and thus there is, in general, considerable reluctance to accept that* B. catarrhalis *may be a pathogen when it is seen. Hence, it is under-reported or totally ignored though there is more awareness regarding its pathogenic potential, particularly as an increasingly high incidence of β lactamase producing strains is being reported from many countries. The importance of this development concerns the choice of routine antibiotic therapy as ampicillin, to which this organism was previously sensitive, may no longer be effective.*

ABRÉGÉ

Neisseria meningitidis *et* Neisseria (Branhamella) catarrhalis *se sont acquises une notoriété significative parmi les cocci gram négatifs du nasopharynx auquel on peut attribuer un pouvoir pathogène. Traditionnellement* B. catarrhalis *est considérée comme un commensal du nasopharynx et il existe donc une réticence considérable vis-à-vis de son caractère pathogène. Par conséquent, sa présence est sous-évaluée ou ignorée bien qu'une prise de conscience se fasse vis-à-vis de son pouvoir pathogène en particulier depuis l'augmentation de fréquence des souches productrices de β lactamase dans de nombreux pays. Cette évolution est d'importance pour le choix d'une antibiothérapie de routine car l'ampicilline, à laquelle cette organisme était préalablement sensible, pourrait ne plus être active.*

*, ** Greenbank Drive, Edinburgh EH10 5SB, Scotland.

Reprints: D. T. McLeod.

HISTORY

Micrococcus Branhamella catarrhalis was first isolated in 1896 from the culture of alveolar material of children with bronchopulmonary infection (11). Subsequently, in 1902 Ghon and Pfeiffer (12) isolated the same organism from the sputum of patients with acute bronchitis who in addition had an influenza-like illness and concluded that *M. catarrhalis* should be regarded as a human pathogen. Three years later Dunn and Gordon supported this opinion by reporting an epidemic of influenza-like illness in Hertford when again this organism was isolated (9). Morphological and biochemical differentiation of Gram-negative cocci was achieved by Von Lingelsheim (17) and Kutscher (16) in 1906 and thereby identification of the various species of micrococcus became possible. Kutscher suspected that some Gram-negative diplococci found in the cerebrospinal fluid in cases of meningitis in 1906 may well have been *M. catarrhalis*. These diagnostic difficulties in distinguishing *N. meningitidis* from *M. catarrhalis* were certainly appreciated and carefully studied by Elser and Huntoon in 1909 (10). Later, Holland in 1920 (2) included *M. catarrhalis* in the genus *Neisseria*. Thereafter, *Neisseria catarrhalis* supplanted the original name of

THYROID PROTECTION AFTER A NUCLEAR REACTOR ACCIDENT

SIR,—Your note (May 24, p 1225) claims that "widespread use" was made of iodine tablets after the Chernobyl nuclear accident and you report that the World Health Organisation's European region would welcome information on complications of this treatment. The main complication to be expected in large regions of central Europe with insufficient iodine supply, hyperthyroidism, was not mentioned. Experts from East and West Europe met at the WHO office in Copenhagen on May 6, and agreed that the environmental contamination with radioiodine from Chernobyl did not necessitate the distribution of stable iodine to the general public. Only in Poland were children in certain areas supplied with stable iodine during the first days of the accident. The West German authorities explicitly asked the public to avoid iodine tablets. In this country with endemic goitre there is a further proportion of the population who are euthyroid and have autonomic thyroid tissue; thus, after intake of higher amounts of stable iodine there would be a risk of hyperthyroidism, especially in older people. Some of these cases might even proceed to thyrotoxic crisis, which may result in early death. This represents the main factor to be taken into consideration in the benefit/risk analysis of any mass distribution of stable iodine in countries such as Germany.

Another point is that some people treated with iodine for goitre prophylaxis might interrupt their medication because they do not know that their "micro-iodine" tablets differ from the "macro-iodine" tablets to prevent the uptake of radioiodine, the use of which was not recommended.

Institut für Genetik
und für Toxikologie von Spaltstoffen,
Kernforschungszentrum Karlsruhe,
D-7500 Karlsruhe 1, West Germany

V. VOLF

PERSISTENCE OF COXSACKIE B VIRUS-SPECIFIC IgM

SIR,—Professor Banatvala and his colleagues (June 28, p 1491) report persistent Coxsackie B virus-specific IgM (for at least 6 years and for 20 months) in two patients with recurrent pericarditis. They apparently used an ELISA to measure virus-specific IgM.

We have obtained similar results using the conventional neutralising antibody test after separation of Ig classes by two-step column chromatography. By rigorous controls false-positive IgM tests are excluded.[1] One patient (born in 1915) presented with hypertension and coronary artery disease and became positive for Coxsackie B type 4 neutralising antibodies in September, 1980, with virus-specific IgM that persisted in high titre to at least November, 1985. Antibody tests for other Coxsackie B virus types were negative. Another patient (born 1919) with the clinical diagnosis of myocarditis exhibited Coxsackie B2 IgM antibodies for at least 15 months. A third (born 1946, with cardiac neurasthenia and palpitations) had IgM antibodies to Coxsackie B virus types 2, 4, and 5 for at least 18 months.

HOMOTYPIC AND HETEROTYPIC NEUTRALISING ANTIBODY RESPONSE TO COXSACKIE VIRUS B TYPES 1–6 IN PATIENT WITH ASEPTIC MENINGITIS DUE TO COXSACKIE B TYPE 2

Type	—	Neutralising antibody titre at time (mo): 0	1/2	1	2	6*	7	11
1	IgG	512	256	256	192	1024	768	1536
1	IgM	33	neg	20	22	278	154	220
2	IgG	neg	neg	neg	neg	96	768	192
2	IgM	..	..	..	..	185	116	55
3	IgG	neg	neg	neg	neg	neg	neg	neg
3	IgM	..	..	..	neg	neg	..	..
4	IgG	neg	neg	neg	neg	neg	neg	neg
4	IgM	..	..	..	..	..	..	..
5	IgG	48	96	64	64	384	512	64
5	IgM	..	17	neg	neg	278	58	110
6	IgG	neg	neg	neg	neg	64	24	24
6	IgM	..	..	..	..	46	29	55

*Onset of illness. Coxsackie virus B2 isolated.
neg = negative; .. = not done.

However, we hesitate to interpret the persistence of Coxsackie B IgM as resulting generally from prolonged antigenic stimulation by persisting infection (whole virus or some of its components), though Dr Bowles and colleagues' report (May 17, p 1120) of Coxsackie B RNA sequences in the myocardium is compatible with this view. We have seen an apparently healthy 33-year-old man with Coxsackie B type 1 IgM persisting for at least 6 months (table). At that time he fell ill with aseptic meningitis due to Coxsackie B virus type 2 (virus isolated). He developed not only Coxsackie B type 2 IgM but also seroconverted to Coxsackie B type 6 (IgG and IgM) and had a rise in IgM of at least 10-fold to Coxsackie B types 1 and 5. IgM against these four Coxsackie B virus types persisted for at least 5 months without a significant decline of titre.

We cannot exclude persistence of Coxsackie B viruses in these patients. But from a practical point of view we should like to emphasise that Coxsackie B IgM may persist for long periods of time and that significant heterotypic responses may be observed.[2,3] The difficulty is to recognise recent infections and to establish a type-specific diagnosis.

Institute for Virology,
University of Cologne,
D-5000 Cologne 41, West Germany

H. J. EGGERS
TH. MERTENS

1. Mertens Th, Hager H, Eggers HJ. Epidemiology of an outbreak in a maternity unit of infections with an antigenic variant of echovirus 11. *J Med Virol* 1982; 9: 81–91.
2. Mertens Th, Pika U, Eggers HJ. Cross antigenicity among enteroviruses as revealed by immunoblot technique. *Virology* 1983; 129: 431–42.
3. Eggers HJ, Mertens Th. Viruses and myocardium: Notes of a virologist. *Eur Heart J* (in press).

NON-SECRETION OF ABO ANTIGENS PREDISPOSING TO INFECTION BY NEISSERIA MENINGITIDIS AND STREPTOCOCCUS PNEUMONIAE

SIR,—A 1985 *Lancet* editorial emphasised the need for research on vaccines for serogroup B *Neisseria meningitidis*.[1] The absence of serogroup-specific antibodies is an important factor predisposing the host not only to meningococcal infections but also to those caused by *Streptococcus pneumoniae*.[2,3] Our work on other infectious agents suggested that factors that might be associated with susceptibility of the non-immune host should be examined. Among patients with recurrent urinary infections[4,5] or superficial infections due to *Candida albicans*[6,7] we found a significant increase in the prevalence of non-secretion of ABO blood group antigens into body fluids. Secretion of the water-soluble glycoprotein form of the ABO antigens is inherited in a mendelian-dominant pattern. The frequency of non-secretors was especially high among patients with candida infections in whom the specific immune responses might be considered to be immunocompromised, and in pregnant women with vaginal infection and elderly patients of both sexes with oral infections.[7] Secretion of blood group antigens appears to play a part in the innate defences at the mucosal surfaces. Because they lacked protective antibodies, we viewed non-immune individuals as immunocompromised hosts at the time they were exposed to the pathogenic meningococci or pneumococci. As a result, we predicted there would be a higher incidence of non-secretors among patients with these infections.

Saliva specimens were boiled within 3 h of collection and stored at −20°C. Secretor state was determined by the haemagglutination inhibition assay[8] and the results were compared with figures for blood donors in the Edinburgh area[4] and for blood donors and hospital staff in Iceland.[9]

Non-secretor status was significantly more frequent in the Scottish patients with pneumococcal disease and with meningococcal disease and in the Icelandic patients with meningococcal infections (table 1).

Andersson and co-workers[10] have reported significantly decreased attachment of pneumococci to epithelial cells when bacteria were preincubated with glycocompounds found in pooled human milk. Since human milk is a rich source of blood group substances, our epidemiological studies complement their in vitro observations.

TABLE I—INCIDENCE OF NON-SECRETORS AMONG PATIENTS WITH INFECTIONS DUE TO N MENINGITIDIS AND STREP PNEUMONIAE

Infection	Source	Non-secretors	p
N meningitidis	Scotland	18/26 (69%)	< 0·0005
	Iceland	53/98 (54%)	< 0·05
Str pneumoniae	Scotland	22/47 (47%)	< 0·01
Controls	Scotland*	89/334 (26·6%)	
	Iceland*	94/228 (41·2%)	

TABLE II—BLOOD GROUP GLYCOPROTEINS PRESENT IN SECRETOR AND NON-SECRETOR BODY FLUIDS.

	A*	B	H†	Lewis‡	Lewis‡
Secretor	+/−	+/−	+	±‡	+
Non-secretor	−	−	−	+	−

*Depending on presence of A or B blood group genes.
†H antigen is antigen of blood group O.
‡Present in small quantities as precursor of Lewis^b.

Our work on candida suggested two mechanisms by which the glycocompounds in the body fluids of secretors and non-secretors (table II) might inhibit or enhance binding of microorganisms to epithelial cells. Boiled saliva from secretors of blood groups A, B, and O can inhibit binding of the yeast to buccal epithelial cells.[6,7] Saliva of non-secretors enhanced the binding.[6,7] We have found that the Lewis' antigen in the body fluids of non-secretors is one of the receptors for *C albicans*.[11] Unlike ABO blood group antigens, Lewis antigens are not structural components of host cells. They are acquired as a coat from either plasma or secretions. If the carbohydrate moiety of the Lewis antigen in secretions recognises an adhesin on the surface of a bacterium or yeast, it might bind afterwards to the epithelial surface by another portion of the molecule along with the attached microorganism.

Investigations are underway to find out if similar host-parasite interactions might account for the increased incidence of non-secretors among patients with infections due to *N meningitidis* and *Strep pneumoniae*. Identification of the receptor for these organisms would facilitate isolation of adhesins involved in colonisation of the mucosal surfaces and their assessment as potential vaccine candidates.

We thank the Edinburgh and South East Scotland Regional Blood Transfusion Service for the reagents used in this study.

Department of Bacteriology, Medical School, University of Edinburgh, Edinburgh EH8 9AG — C. C. BLACKWELL

Department of Bacteriology, University of Iceland, Reykjavik, Iceland — K. JÓNSDÓTTIR

Central Microbiological Laboratories, Edinburgh — M. HANSON

Infectious Diseases Unit, Monklands District General Hospital, Airdrie — W. T. A. TODD, A. K. R. CHAUDHURI, B. MATHEW

Infectious Diseases Unit, City Hospital, Edinburgh — R. P. BRETTLE

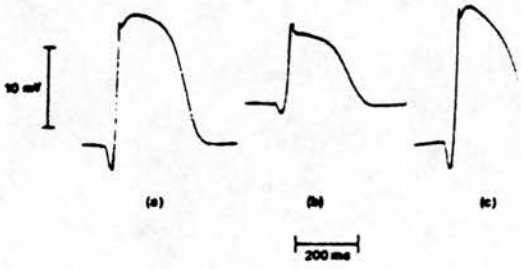
Department of Bacteriology, Medical School, Edinburgh — D. M. WEIR

1. Editorial. Defence against meningococcal disease. *Lancet* 1985; ii: 929–30.
2. Greenwood BM. Selective primary health care: Strategies for control of disease in the developing world XIII: Acute bacterial meningitis. *Rev Infect Dis* 1984; 6: 374–89.
3. Brunsen LA, Norrby R, Trollfors B. Invasive pneumococcal infections: Incidence, predisposing factors and prognosis. *Rev Infect Dis* 1985; 7: 133–42.
4. Kinane DF, Blackwell CC, Brettle RP, Weir DM, Winstanley FP, Elton RA. ABO-blood group, secretor state and susceptibility to recurrent urinary tract infection in women. *Br Med J* 1982; 285: 7–9.
5. Blackwell CC, May SJ, Brettle RP, MacCallum CJ, Weir DM. Host-parasite interactions underlying non-secretion of blood group antigens and susceptibility to recurrent urinary tract infections in women. In: Lark D, ed. Protein-carbohydrate interactions in biological systems. London: Academic Press (In press).
6. Blackwell CC, Thom SM, MacCallum CJ, Weir DM, Kinane DF, Johnstone FD. Host-parasite interactions underlying non-secretion of blood group antigens and susceptibility to infection by *Candida albicans*. In: Lark D, ed. Protein-carbohydrate interactions in biological systems. London: Academic Press (In press).
7. Blackwell CC, Thom SM, Lawrie OR, Weir DM, Wray D, Ka... Non-secretion of blood group antigens and susceptibility to oral ca... *Candida albicans*. *J Dent Res* (in press).
8. Mollison PL. Blood transfusion in clinical medicine. 6th ed. Oxford: Scientific Publications, 1979: 414–82.
9. Thorderson G, Bjarnason O, Lincoln PJ, Dodd BE. ABH secretor and ... frequencies in an Icelandic series. *J Med Genet* 1972; 9: 46–47.
10. Andersson B, Porras O, Hanson LÅ, Lagergård T, Svanborg-Edén C. In... attachment of *Streptococcus pneumoniae* and *Haemophilus influenzae* by h... and receptor oligosaccharides. *J Infect Dis* 1986; 153: 232–37.
11. May SJ, Blackwell CC, Weir DM. Non-secretion of blood group ... susceptibility to *Candida albicans*: the role of Lewis group antigens. *J D...* press).

EPICARDIAL MONOPHASIC ACTION POTENT

SIR,—We agree with Dr Taggart and colleagues (Ju... p 1462) that epicardial monophasic action potential recordin... considerable promise. We have devised a similar technique[1] ... studying 37 patients, have concentrated on the error ... reproducibility of the method. Our experience shows the n... caution in interpretation.

We have found the amplitude of monophasic action poten... be of limited value. Amplitude is critically dependent ... pressure and orientation of the electrode. Gross changes ... observed, within a short time interval, independently ... intervention. This is illustrated in the figure. Potentials (a), (... (c) were recorded from the same epicardial site over ... Considerable variation in amplitude is obvious. Taggar... observed potentials for 30 s before the induction of ischaem... attempt to ensure stability, but this is no guarantee ... subsequent deterioration in the signal. Demonstration of cor... recovery of the potentials in all patients, on restoration of ... would have strengthened their conclusions.



Variation in epicardial monophasic action potential.

We believe that the technique provides a valid measure of ac... potential duration, but again limitations should be recognised. ... 95% confidence limits for our replicate observations of dura... have varied between 24 and 40 ms in different patients. ... compares with a mean difference of 25 ms between ischaemic ... non-ischaemic periods reported by Taggart et al. Hence ... reported ischaemic changes are of a magnitude which border on ... resolution of the method. In addition, we have evidence ... monophasic potentials are influenced by temperature and by ... institution of cardiopulmonary bypass.

It is important that the limitations of any new technique sh... be recognised. We suggest that this is especially relevant in rela... to epicardial monophasic potentials.

Department of Cardiology, Freeman Hospital, Newcastle upon Tyne NE7 7DN — J. C. COWAN, C. J. HILTON, C. J. GRIFFITHS, R. W. F. CAMPB...

1. Cowan JC, Hilton CJ, Griffiths CJ, Bourke J, Tamaphaewdigul S, Mann... Campbell RWF. Epicardial repolarisation mapping in man. *Br Heart J* 198... 534P.

PSP may be a sensitive marker for pancreatic inflammation, reflecting pancreatic acinar cell damage persisting after acute pancreatitis and in chronic pancreatitis. We are now correlating PSP levels with other diagnostic procedures.

W.-H. SCHMIEGEL
M. BURCKERT
H. KALTHOFF
H. G. THIELE
H. G. BÖTZOW
G. KLOSE
H. GRETEN

Medical Clinic,
University of Hamburg,
Hamburg, West Germany

1. Giorgi D, Bernard JP, De Caro A, et al. Pancreatic stone protein I: Evidence that it is encoded by a pancreatic messenger ribonucleic acid. *Gastroenterology* 1985; 89: 381–86.
2. Figarella C, Amouric M, Guy-Crotte O. Proteolysis of human trypsinogen I: Pathogenic implication in chronic pancreatitis. *Biochem Biophys Res Commun* 1984; 118: 154–61.
3. Mouratis G, Lumbar M, De Caro A, Malagnac L, Sarles H, Delmas M. Analysis of human pancreatic juice constituents related to the pancreatic stone protein using monoclonal anybody. *CR Acad Sci (Paris)* 1985; 300: 199–202.

NON-SECRETION OF ABO BLOOD GROUP ANTIGENS PREDISPOSING TO INFECTION BY HAEMOPHILUS INFLUENZAE

SIR,—We have reported (Aug 2, p 284) a significant increase in the incidence of non-secretors of ABO blood group antigens among patients with infections due to *Neisseria meningitidis* or *Streptococcus pneumoniae*. We suggested that the ability to secrete blood group antigens plays a part in the innate defences of the non-immune host against these infectious agents. These two organisms and *Haemophilus influenzae* are responsible for 75% of bacterial meningitis in young children during the period in which maternal antibodies have waned and the infant has not developed active immunity.[1] If secretion plays a similar protective role in the host-parasite interactions that lead to establishment or prevention of these infections, we predicted an increased incidence of non-secretors among children with haemophilus infections.

Saliva samples were obtained from 43 Icelandic children who had invasive disease due to *H influenzae* (meningitis 30, sepsis/cellulitis 11, and epiglottitis 2). Secretor state was assessed by the haemagglutination inhibition method.[2] The saliva specimens were boiled within 2 h of collection and stored frozen until tested. The secretor states of the patients were compared by χ^2 test (with Yates' correction) with those reported for Icelandic blood donors and hospital personnel.[3]

29/43 patients (67%) and 94/228 (41%) controls[3] were non-secretors ($p < 0.005$). These findings confirmed our prediction that, as we had found for pneumococcal and meningococcal infections, the inability of an individual to secrete his ABO blood group antigens is a factor that predisposes to infection with haemophilus. Secretor state is a stable host factor that can be used in investigations of the pathogenesis and development of methods for prevention of these paediatric infections.

We thank the Edinburgh and South East Scotland Blood Transfusion Service for reagents used in this study.

Department of Bacteriology,
Medical School,
University of Edinburgh,
Edinburgh EH8 9AG
C. C. BLACKWELL

Department of Bacteriology,
University of Iceland,
Reykjavik, Iceland
K. JONSDOTTIR

General Microbiological Laboratories,
Western General Hospital,
Edinburgh
M. F. HANSON

Department of Bacteriology,
Medical School,
University of Edinburgh
D. M. WEIR

1. Greenwood B. Selective primary health care: Strategies for control of disease in the developing world XIII: acute bacterial meningitis. *Rev Infect Dis* 1984; 6: 374–89.
2. Mollison PL. Blood transfusion in clinical medicine, 6th ed. Oxford: Blackwell Scientific Publications, 1979: 414–82.
3. Thorderson G, Biarnason O, Lincoln PJ, Dodd BE. ABH secretor and Lewis type frequencies in an Icelandic series. *J Med Genet* 1972; 9: 46–47.

MATERNAL SMOKING DURING PREGNANCY AND THE RISK OF CHILDHOOD CANCER

SIR,—We are responding to three of the letters in your Aug 30 issue (pp 519 and 520) commenting on our June 14 paper. The discrepancy between our findings and those from the Inter-Regional Epidemiological Study of Childhood Cancer (IRESCC) is attributed by Dr McKinney and Dr Stiller to differences in study design. In IRESCC two children were age and sex matched for every case but we are not convinced that individual matching is a safeguard against recall bias, especially if diseases with totally different outcomes are being compared. It is unlikely that mothers of children admitted with, say, accidental injury or for tonsillectomy, will try to recall past events as vigorously as will mothers of children with a malignant or serious, chronic disease of unknown origin. Furthermore, controls selected from general practitioner lists and hospital admissions for minor conditions may be biased in respect of smoke exposure. There are several reports of an association between maternal smoking and, for example, upper-respiratory-tract disease, bronchitis, pneumonia, asthma, coughs, and adenoidectomy/tonsillectomy in their children. This may partly explain why the IRESCC found no evidence of an increased risk of cancer in the children of mothers who smoked during pregnancy.

Diabetic children are not representative of the general population of children but that does not necessarily mean that the smoking histories for their mothers are atypical. The smoking habits of the mothers we studied correspond fairly well with those of women of similar ages in the general population in Sweden[1] (table I), and it is clear that mothers of children with leukaemia smoked more than the average.

McKinney and Stiller also raise the question of different ages at diagnosis in cases and controls, and hence different intervals since the pregnancy. Age-specific confounding was controlled for by principles outlined by Miettinen[2,3] and by calculating the confidence interval (CI) of the risk ratio.[4] Indirect standardisation was done with the age-specific strata 0–4, 5–9, and 10–16. In the acute lymphoblastic leukaemia (ALL) *vs* diabetes setting this gave, for the group exposed to 10 or more cigarettes per day, a standard morbidity ratio (SMR) of 2·03; the crude risk ratio (CRR) was 2·07.

TABLE I—PREVALENCE OF DAILY CIGARETTE SMOKING AMONG MOTHERS OF CASES AND CONTROLS AND OF WOMEN IN CHILDBEARING AGES IN SWEDEN

Group	Daily cigarette smokers (%) among mothers born in:		
	1926–35	1936–45	1946–58
ALL*	20·0	60·7	62·7
Solid tumours*	30·0	42·4	46·3
Diabetes* (controls)	29·8	41·7	48·1
Twin study[1]	29·4	39·0	40·4

*Data relate to 5-year period before pregnancy.

TABLE II—ACUTE LYMPHOBLASTIC LEUKAEMIA ACCORDING TO MATERNAL SMOKING AND OCCUPATION DURING PREGNANCY

Occupation*	Exposed/unexposed in:		CRR	95% CI
	Cases	Controls		
Housewife	19/32	51/108	1·26	0·65–2·43
Administration, office	7/10	14/34	1·70	0·54–5·38
Nurse, nurse assistant	9/9	8/24	3·00	0·89–10·10
Teacher	4/7	1/16	9·14	1·07/78·30
Industrial, construction	8/2	5/10	8·00	1·30/49·28
Service	7/4	5/8	2·80	0·52–14·98
Commercial, sales, sales staff	3/5	5/6	0·72	0·11–4·85
Technical, scientific	0/4	1/8	0	0
Agriculture, forestry, fishing	0/1	2/2	0	0
Transport, communication	0/0	1/2	—	—
Student	0/1	0/2	—	—

*Classified according to the National Central Bureau of Statistics (POB, 1980).

J. Clin. Lab. Immunol. (1987), 22, 133–137

SECRETOR STATE AND IMMUNOGLOBULIN LEVELS AMONG WOMEN WITH RECURRENT URINARY TRACT INFECTIONS

C. C. BLACKWELL,[1] S. J. MAY,[1] R. P. BRETTLE,[2] C. J. MACCALLUM[1] and D. M. WEIR[1]
[1]*Department of Bacteriology, University of Edinburgh, Medical School, Teviot Place and* [2]*Pyelonephritis Unit and Division of Infectious Diseases, City Hospital, Greenbank Drive, Edinburgh, UK*
(Received 29 July 1986)

SUMMARY In this study we tested the hypothesis that the increased susceptibility of non-secretors of blood group antigens to recurrent urinary tract infections might be due, in part, to lower levels of IgA reported for non-secretor Caucasian women. Total serum IgA and IgG levels of 200 women in a retrospective study group monitored by the local pyelonephritis unit for 20 yr was compared with those of 100 women from a prospective group recently referred to the clinic.

Immunoglobulin levels of the retrospective group were analyzed with reference to the secretor state of the patient, improvement over the 20 yr and presence or absence of renal scars. Total IgA and IgG levels were significantly higher in the sera of non-secretors with or without renal scars and also among patients who had clinically improved over the 20-yr period. Most women appeared to improve regardless of age of onset of infections or treatment prescribed. It was suggested that improvement might be associated with a self-immunization phenomenon. The significantly higher levels of IgA and IgG found among the women in the retrospective study compared with the levels found in the sera of age-matched women in the prospective study is evidence for this hypothesis. The implications of these findings for further investigations of the pathogenesis of urinary tract infections are discussed.

Key words: Urinary tract infections, secretor state, immunoglobulins, women

INTRODUCTION

RECURRENT urinary tract infections (UTI) occur among women despite the availability of antibacterial agents with good *in vitro* efficacy. Up to 80% of these recurrent infections have been found to be reinfections, suggesting that there may be a failure of host defences in this population (1). Non-secretion of ABO blood group antigens is a stable host marker associated with susceptibility of non-immune hosts to meningococcal and pneumococcal infections (2). We have also found a higher incidence of non-secretors in adult women with recurrent urinary tract infections (3, 4) and non-secretion appears to predispose the host to kidney scarring associated with pyelonephritic infections (Lomberg and Svanborg-Eden, personal communication; May *et al.*, manuscript in preparation).

Indirect evidence from early epidemiological observations by groups working on problems unrelated to urinary tract infections led us to examine the hypothesis that the reported lower levels of serum IgA found among non-secretor women might play a role in host-parasite interactions underlying their increased susceptibility to recurrent UTI. In a randomly selected population, Caucasian women who were non-secretors had lower levels of serum IgA than secretors (5). A similar pattern had also been reported for secretory IgA levels. Those of non-secretors were lower than those of secretors (6).

Among women of child-bearing age, isolation of *Candida albicans* has been associated with low levels of specific anti-candida serum IgA but not anti-candida serum IgG antibodies (7). If a similar set of interactions occurs at the mucosal surfaces of the urinary tract, the apparent increased susceptibility of non-secretors to recurrent UTI might be due, in part, to low levels of serum IgA specific for the infecting strain of *Escherichia coli*. Secretory IgA levels have been found to be lower in young girls with recurrent urinary tract infections compared with levels for age matched controls (8).

Because there is no one bacterial strain or common antigen to which protective antibodies have been shown to be produced (9–12), total serum IgA and IgG levels were determined for 2 groups of patients: a retrospective study group, women who had been monitored for 20 yr

Correspondence: Dr. C. C. Blackwell, Department of Bacteriology, University of Edinburgh, Medical School, Teviot Place, Edinburgh, UK.

by the pyelonephritis clinic; and a prospective study group, women recently referred to the clinic for investigation of recurrent UTI. We predicted that if non-secretors were not able to produce an effective IgA response to mucosal pathogens their total serum IgA would be lower than that of secretors. Analysis of our results in the context of severity of the disease and clinical improvement over 20 yr provided insights into the roles of both innate factors and specific immune responses in the host's defences against these infections. The implications of these findings for further investigations of the pathogenesis of UTI are discussed.

Patients and Methods

Patients in the study had been referred to the Pyelonephritis Clinic, City Hospital, Edinburgh for specialist investigation of recurrent urinary tract infection. There were 2 groups: a retrospective group consisting of 202 women who had been monitored for 20 yr by the clinic; and 100 patients selected from a prospective study group of 516 women referred during or after 1982 to the Pyelonephritis Unit for investigation of recurrent UTI. The 100 women from the prospective study were selected by age to compare the immunoglobulin levels of women who had recently developed recurrent urinary tract infections with those found for women who had these infections for a number of years.

The records of 200 patients in the retrospective study were assessed to determine diagnosis and clinical progress over 2 decades. Of these, 54 had renal scars as defined by pathological examination of nephrectomy specimens (19 patients) or radiological evidence (35 patients). The remainder had a history of recurrent symptomatic infections but no radiological evidence of scarring. The mean age of the women with renal scars was 50·5 yr (range 23–74) and of the women without scars 54 yr (range 27–84). The differences in ages were not statistically significant (13).

Improvement was defined as fewer episodes of infection in the second decade of the study compared with the number in the first decade. No improvement was defined as the same number or more infections in the second decade. For the group with renal scars the mean number of documented infections for 42 patients during the first decade was 5 (range 1–20) and for the second decade 2·5 (range 0–10). For the group without renal scars the mean number of documented infections for 96 patients for the first decade was 4·6 (range 0–23) and for the second decade 2·8 (range 0–35). The differences between the episodes of infection per decade were 2·4 for the patients with renal scars and 1·8 for those without scars ($p < 0·001$ for both groups) (13).

Secretor state was determined by the method described by Mollison (14) and total serum IgA and IgG levels determined with NOR-Partigen immunodiffusion plates (Behring Diagnostics). Data from those patients between the ages of 23 and 64 were used in the analyses of improvement and diagnostic category because the age range for which the plates were standardized is 16–64. The results were expressed as international units per ml and analyzed by X^2 or Student's t-test.

Results

Secretor State of Patients in the Study

Compared with local controls (26·6%), there was a significant increase in the incidence of non-secretors (34·8%, $p < 0·01$) among the 718 women referred to the clinic. This increase in non-secretors was found in both the prospective and retrospective groups.

Table 1 Incidence of non-secretors among women referred for investigation of recurrent urinary tract infection

	No. in each category				
		non-secretors			
Category	*Total*	*no.*	*(%)*	X^2	*p*
Total	718	250	34·8	7·05	<0·01
prospective group	516	172	33·3	4·26	<0·05
retrospective group	202	78	38·6	7·856	<0·01
without renal scars	146	53	36·6	4·094	<0·05
with renal scars	54	23	42·6	5·006	<0·05
improved	127	47	37	4·264	<0·05
not improved	50	20	40	3·186	n.s.
no change	22	10	45·5	2·760	n.s.
Controls	334	89	26·6		

Among the 202 patients in the retrospective study, 78 (38·6%) were non-secretors. This significantly increased incidence of non-secretors was observed among the women with renal scarring and those with no evidence of scars (42·6% and 36·6% respectively) and those who had improved over the 20 yr (37%). The remaining groups showed a similar pattern, but the numbers were too small to provide statistically significant differences (table 1).

Total Serum Immunoglobulin Levels

Contrary to our predictions based on the report of Grundbacher (5), there were significantly higher levels

Table 2 Comparison of geometric means of total serum IgA and IgG levels from secretors and non-secretors in the different diagnostic categories

Category	*No.*	*IgA IU/ml*	*p*	*IgG IU/ml*	*p*
Total patients					
secretor	111	103	<0·01	118	<0·0025
non-secretor	70	125·4		139·7	
without renal scars					
total	131	115·9		128·9	
secretor	82	107·9	<0·05	123·3	n.s.
non-secretor	49	127·4		138·7	
with renal scars					
total	48	101·7		120·2	
secretor	29	90·3	<0·0025	104·3	<0·025
non-secretor	19	121·8		151	
improved					
total	118	110·9		125·5	
secretor	74	99·3	<0·0025	117·2	<0·01
non-secretor	44	133·1		140·7	
not improved					
total	40	114·3		128·8	
secretor	24	115·1	n.s.	125·4	n.s.
non-secretor	16	113·3		133·8	
no change					
total	20	110		129·4	
secretor	11	108	n.s.	114·5	n.s.
non-secretor	9	112		153·1	

Table 3 Comparison of geometric means of serum immunoglobulin levels between patients in the retrospective and prospective studies

	Geometric means of serum immunoglobulin levels					
Patient category	*no.*	*IgA IU/ml*	*p*	*no.*	*IgG IU/ml*	*p*
Total						
retrospective	182	111·9	<0·001	182	126·2	<0·001
prospective	100	82·4		100	105·7	
secretors						
retrospective	111	103	<0·01	111	118	n.s.
prospective	65	82·2		65	108	
non-secretors						
retrospective	71	127·1	<0·001	71	146	<0·001
prospective	35	89·7		35	104	

of IgA and also IgG among the non-secretors in the retrospective study. This increase was observed in each of the categories in Table 1 in which there was a significant increase in the incidence of non-secretors (table 2). The levels of immunoglobulins found among patients in the prospective study were significantly lower than those found for age-matched women in the retrospective study. In the prospective study there were no significant differences in the levels of IgA or IgG of secretors and non-secretors (table 3). Comparison of IgA and IgG levels between these 2 groups by decade of birth is shown in Table 4.

When immunoglobulin levels for the patients in the retrospective study without renal scars were analyzed with reference to improvement, there was a significant difference between the IgA levels of secretors and non-secretors who had improved; however, these differences were not apparent for any of the other categories or for IgG levels. The mean of the IgA levels for non-secretors who had not improved (106 IU/ml) was lower than that for non-secretors who had improved (136 IU/ml). When analyzed by the Student's t-test, these differences were just outside the 95% confidence limits (table 5).

Table 6 presents the means of the immunoglobulin levels for patients with renal scars. For the non-secretors who had improved, both IgA and IgG levels were significantly higher than those for secretors who had improved. Among these patients who had not improved, the IgG levels of secretors were significantly lower than those for non-secretors. The IgA levels for secretors who had improved (83·9 IU/ml) were lower than those for secretors who had not improved (128·9 IU/ml), but these differences were just outside the 95% confidence limits.

Table 4 Comparison of geometric means of total serum IgA and IgG of patients in retrospective study with those from women in the prospective study matched by decade of birth

	Retrospective study		*Prospective study*		
Decade of birth	*no.*	*IgA IU/ml*	*no.*	*IgA IU/ml*	*p*
1900–1910	7	133·3	4	131	n.s.
1911–1920	30	107·9	11	108·1	n.s.
1921–1930	43	112·2	16	68·5	<0·0025
1931–1940	69	110·7	23	81·1	<0·0125
1941–1950	17	109·6	23	75·7	<0·01
1951–1960	12	130·6	15	95·1	<0·01
1961–1970	3	87·1	9	69·7	n.s.
		IgG IU/ml		*IgG IU/ml*	*p*
1900–1910	7	103	4	110·7	n.s.
1911–1920	30	120·5	11	113·5	n.s.
1921–1930	43	127·1	16	101·4	<0·025
1931–1940	70	125·9	23	105·7	<0·025
1941–1950	17	137·4	23	107·4	<0·005
1951–1960	11	132·1	15	102·1	<0·025
1961–1970	3	158·1	9	109·9	n.s.

Table 5 Comparison of geometric means of total serum IgA and IgG levels from secretors and non-secretors without renal scars with reference to improvement over 20 yr

Category	*No.*	*IgA IU/ml*	*p*	*IgG IU/ml*	*p*
improved	81	117·8		127·5	
secretor	50	107·8	<0·05	121·3	n.s.
non-secretor	31	136		138	
not improved	32	109		135·6	
secretor	19	111·6	n.s.	137·3	n.s.
non-secretor	13	106		130·7	
no change	16	116		129	
secretor	11	107·9	n.s.	114·5	n.s.
non-secretor	5	136		168·3	
Mean value (Healthy Central Europeans 15–64 years old)		125		144	

Table 6 Comparison of geometric means of total serum IgA and IgG levels of secretors and non-secretors with renal scars with reference to improvement over 20 yr

Category	No.	IgA IU/ml	p	IgG IU/ml	p
improved	36	96·7		123·2	
secretor	24	83·9	<0·01	109·1	<0·01
non-secretor	12	128·5		156·9	
not improved	8	136·3		104·3	
secretor	5	128·9	n.s.	84·1	<0·05
non-secretor	3	149·5		149·4	
no change	4	88·8		130·8	
secretor	0	—		—	
non-secretor	4	88·8		130·8	
Mean value (Healthy Central Europeans 15–64 years old)		125		144	

The differences between IgG values for secretors who had improved and those who had not were not significant.

Discussion

The results refute our original hypothesis that the increased incidence of non-secretors among women with recurrent UTI might be associated with an inability to produce an IgA response as effectively as secretors. The total serum IgA levels of the non-secretors in the retrospective study were significantly higher than those for secretors as were their IgG levels. These higher levels of immunoglobulins were found for non-secretors regardless of the presence or absence of renal scars. The raised levels of these antibodies among non-secretor patients who had improved suggests that non-secretors are more dependent on their specific immune responses for protection against these infections.

The IgA and IgG levels of women recently referred to the clinic were significantly lower than those of patients of similar ages in the retrospective group (table 3). There were no significant differences in either IgA or IgG levels of the secretors and non-secretors in the prospective study group. If initial IgA levels of non-secretors were lower than secretors as suggested by Grundbacher (5), these results might be due to responses to the antigenic stimuli of their infecting strains. Any initial differences in immunoglobulin levels are not found by the time these women have suffered enough episodes of UTI to be referred for specialist investigation.

Regardless of age of onset or therapy prescribed, the majority of the women followed for 20 yr by the clinic had fewer episodes of infection during the second decade. This occurred in women who had begun to have symptoms at a very early age as well as those whose problems began with the onset of sexual activity. The decrease in the numbers of infectious episodes was not associated solely with a decrease in frequency of intercourse. This suggested a self-immunization phenomenon. Comparison of the total serum IgA and IgG levels of the women in the retrospective study with those found for women of the same ages recently referred to the clinic supported this hypothesis (tables 3, 4). The significantly higher levels of total serum IgA and IgG among the retrospective group suggests that antibody response might play a part in controlling recurrent infections. A significant correlation between the levels of anti-candida IgA and IgG in serum and cervico-vaginal secretions has been reported (15). If similar responses to infecting strains of *E. coli* are elicited, the increased levels of serum IgA and IgG might reflect increased levels of these antibodies in body fluids and secretions.

When the immunoglobulin levels were examined with reference to improvement, the pattern for patients without renal scars differed from that observed for those with scarring. Among patients without scars, the only significant difference found between secretors and non-secretors was an increase in the total serum IgA levels of non-secretors who had improved. These findings appear to parallel those of the association between the decreased incidence of carriage of *C. albicans* with high levels of specific anti-candida serum IgA. Our next step in elucidation of protective immune response to UTI is identification of the antigen(s) that have elicited these IgA antibodies in the sera of women who have improved.

Among women with scars who had improved, there was a similar significant difference in total IgA in the sera of non-secretors compared with secretors. Non-secretors in this category also had higher levels of IgG. Among the patients with scars who had not improved, there was an intriguing difference in the levels of IgG found for secretors and non-secretors. Although the numbers of patients were small, the IgG levels of 4 of the 5 secretors were significantly lower than those for the non-secretors in the study and unusually low compared with the reported mean for central Europeans (16), 144 IU/ml: 74·8, 70·5, 63·8 and 80·5 IU/ml.

A recent report described lung injury mediated by reactive oxygen radicals produced by tissue macrophages that had bound immune complexes containing IgA (17). There is a significant increase in the incidence of non-secretors among children who develop renal scarring as a result of recurrent urinary tract infections. If the immune responses in children parallel those we have observed in adults, the following scheme might partly explain the association between non-secretion and formation of renal scars in children.

The uroepithelial cells of non-secretors have been shown to bind significantly higher numbers of uropathogenic bacteria than secretors (18). If this increases the probability of colonization the following interactions might occur. In the non-immune non-secretor the infection would be countered only by the inflammatory

response. This might result in tissue damage if the organism reached the kidney. There is evidence that IgA is produced locally in the lower urinary tract in response to infecting bacteria. Secretory IgA appeared in the urine of some children with pyelonephritis and most of the patients with cystitis before antibodies were detected in the serum (19). If complexes of bacteria and IgA were to be formed in the kidney, these might induce tissue damage by mechanisms analogous to those reported in the lung (17). High levels of IgG antibodies are also found in the urine and a similar mechanism for tissue damage mediated by IgG immune complexes and polymorphonuclear leukocytes has been reported (20).

In conclusion, our results indicate that the increased incidence of non-secretors among patients with recurrent UTI is not associated with an inability to produce an effective humoral response. Non-secretors appear to be more dependent than secretors on their specific immune responses for defence against recurrent UTI because the non-specific protection associated with the secretor gene is absent. The results also suggest an approach to defining the antigens that elicit a protective response in women with recurrent infections and an approach to investigation of the pathogenesis of kidney scarring.

ACKNOWLEDGEMENTS

We are grateful to Dr. J. A. Gray and Dr. P. Welsby for access to their patients and for their support in this project, to Dr. M. Calder and Dr. B. Watt and the staff of the diagnostic laboratories of the City Hospital for their assistance, to the Edinburgh and South-East Scotland Blood Tranfusion Service for reagents used in the study, and to Mrs. M. Cole for her assistance in typing the manuscript. The project was supported by a grant from the Scottish Home and Health Department (K/MRS/50/C449).

REFERENCES

1. Kunin, C. (1970). A ten-year study of bacteriuria in schoolgirls. Final report of bacteriological, urological and epidemiological findings. *J. Infect. Dis.*, 122, 382–393.
2. Blackwell, C. C., Jonsdottir, K., Hanson, M., Todd, W. T. A., Chaudhuri, A. K. R., Mathew, B., Brettle, R. P. and Weir, D. M. (1986). Non-secretion of ABO antigens predisposing to infection by *Neisseria meningitidis* and *Streptococcus pneumoniae*. *Lancet*, ii, 284–285.
3. Kinane, D. F., Blackwell, C. C., Brettle, R. P., Weir, D. M., Winstanley, F. P. and Elton, R. A. (1982). ABO blood group, secretor state and susceptibility to recurrent urinary tract infection. *Br. Med. J.*, 285, 7–9.
4. Blackwell, C. C., May, S. J., Brettle, R. P., MacCallum, C. J. and Weir, D. M. (1986). Host-parasite interactions underlying non-secretion of blood group antigens and susceptibility to recurrent urinary tract infections. In *Protein Carbohydrate Interactions in Biological Sciences* (edited by D. Lark), pp. 229–230. Academic Press, London.
5. Grundbacher, F. J. (1972). Immunoglobulins, secretor status and the incidence of rheumatic fever and rheumatic heart disease. *Hum. Hered.*, 25, 399–404.
6. Waissbluth, J. G. and Langman, J. S. (1971). ABO blood groups, secretor status, salivary protein, and serum and salivary immunoglobulin concentrations. *Gut*, 12, 646–649.
7. Schønheyder, N., Johansen, J. A., Møller-Hansen, C. and Sterderup, A. (1983). IgA and IgG serum antibodies to *Candida albicans* in women of child bearing age. *Sabouraudia*, 21, 223–231.
8. Tuttle, J. P., Sarvas, H. and Koistinen, J. (1978). The role of vaginal immunoglobulin A in girls with recurrent urinary infections. *J. Urol.*, 120, 742–744.
9. Silverblatt, F. J. and Cohen, L. S. (1979). Antipili antibodies afford protection against ascending pyelonephritis. *J. Clin. Invest.*, 64, 333–336.
10. Silverblatt, F. J., Weinstein, R. and Rene, P. (1982). Protection from experimental pyelonephritis by antibody to pili. *Scand. J. Infect. Dis.*, 33 (Suppl.), 79–82.
11. Ørskov, I., Ørskov, F., Birch-Anderson, A., Kanamoti, M. and Svanborg-Edén, C. (1982). O, K, H & fimbrial antigens in *Escherichia coli* serotypes associated with pyelonephritis and cystitis. *Scand. J. Infect. Dis.*, 33(Suppl.), 18–25.
12. Abraham, S. N., Babu, J. P., Giampapa, C. S., Hasty, D. L., Simpson, W. A. and Beachey, E. H. (1985). Protection against *Escherichia coli*-induced urinary tract infection with hybridoma antibodies directed against type 1 fimbriae or complementary D-mannose receptors. *Inf. Immun.*, 48, 625–628.
13. Brettle, R. P., MacCallum, C. J., Murdoch, J. McC. and Gray, J. A. (1985). The adult female. A twenty year study of bacteriuria in female patients. In *Microbial Disease in Nephrology* (edited by W. A. Asscher and W. Brumfitt), pp. 257–271. John Wiley & Sons Ltd., New York.
14. Mollison, P. L. (1979). *Blood Transfusion in Clinical Medicine* (sixth edition), pp. 414–482. Blackwell Scientific Publications, Oxford.
15. Gough, P. M., Warnock, D. W., Richardson, M. D., Mansel, N. J. and King, J. M. (1984). IgA and IgG antibodies to *C. albicans* in the tract secretions of women with or without vaginal candidiosis. *Sabouraudia*, 22, 265–271.
16. Becker, W., Rapp, W., Schwick, H. G. and Storiko, K. (1968). Methoden zur quantitativen Bestimmung von Plasmaproteinen durch Immunopräzipitation. *Z. Klin. Chem.*, 6, 113–122.
17. Johnson, K. J., Ward, P. A., Kunkel, R. G. and Wilson, B. S. (1986). Mediation of IgA induced lung injury in the rat. *Lab. Invest.*, 54, 499–506.
18. Lomberg, H., Cedergren, B., Leffler, H., Nilsson, B., Carlström, A.-S. and Svanborg-Edén, C. (1986). Influence of blood group on the availability of receptors for attachment of uropathogenic *Escherichia coli*. *Inf. Immun.*, 51, 919–926.
19. Sohl-Akerlund, A., Ahlstedt, S., Hanson, L. A. and Jodal U. (1979). Antibody responses in urine and serum against *Escherichia coli* 0 antigen in childhood urinary tract infection. *Acta Path. Microbiol. Scand.*, 87, 29–36.
20. Johnson, K. J., Wilson, B. S., Till, G. O. and Ward, P. A. (1984). Acute lung injury in rat caused by IgA immune complexes. *J. Clin. Invest.*, 74, 358–369.

Teviot-Kimpton Publications
31 Montpelier Park
Edinburgh EH10 4LX, UK

Non-secretion of ABO blood group antigens as a host susceptibility factor in the spondyloarthropathies

RUTH SHINEBAUM, C CAROLINE BLACKWELL, PETER J G FORSTER, NIGEL P HURST, DONALD M WEIR, GEORGE NUKI

Abstract

Gram negative bacteria precipitate reactive arthritis and may be concerned in the pathogenesis of ankylosing spondylitis and other spondyloarthropathies. Susceptibility to many infectious agents is associated with ABO blood group or secretor state, or both. The distribution of the ABO blood group or secretor state, or both, was therefore determined in 87 patients with ankylosing spondylitis and 32 with other forms of spondyloarthropathy.

The prevalence of non-secretors was significantly increased in the total patient group (54/114; 47%) and in the subgroup with ankylosing spondylitis (41/84; 49%) compared with local controls (89/334; 27%) ($p<0.001$). Other subgroups of patients showed a similarly increased prevalence of non-secretion (33-47%). The distribution of ABO blood groups did not differ between patients and controls.

The association between non-secretor state and ankylosing spondylitis strengthens the hypothesis that ankylosing spondylitis is a form of reactive arthritis. It also suggests several pathogenic mechanisms which may be relevant to the initial host-parasite interaction in ankylosing spondylitis.

Introduction

An infectious aetiology for ankylosing spondylitis has been postulated on several occasions, and many workers consider this disease to be a form of reactive arthritis.[1-3] The hypothesis that the aetiology of ankylosing spondylitis is related to the gut is based on the abnormalities of the bowel that occur in other spondyloarthropathies[4] and on analogy with the reactive arthritis that may occur after gastrointestinal infection.[5] These associations suggest that Gram negative bacteria acting at the gut mucosal surface may be important in the pathogenesis of ankylosing spondylitis. Investigators have examined the role of faecal klebsiella[6,7] and the interaction of these and similar organisms with HLA-B27,[8,9] but no clear relation among enterobacteria, HLA-B27, and ankylosing spondylitis has emerged. It may therefore be pertinent to consider aspects of the host-parasite relation other than HLA-B27, and in particular those host factors associated with infection by Gram negative bacteria.

Blood group specificities of the ABO system are genetically determined, stable host characteristics which have been associated with susceptibility to some infectious diseases. The prevalence of blood group O is increased in patients with typhoid,[10] paratyphoid,[10] and cholera[11]; group B is increased in patients with gonorrhoea,[12] chlamydial infections (unpublished observations), urinary tract infection caused by *Escherichia coli*,[13] and in children with Gram negative enteric infections[14]; and there are reports of an increased prevalence of group A among patients with meningococcal meningitis.[15] By contrast, a normal distribution of blood groups is found among patients with diphtheria, brucellar infections, and whooping cough.[16] At least two infections associated with an increased prevalence of the B blood group, gonorrhoea and chlamydia, may precipitate reactive arthritis.[5]

A second genetically controlled host characteristic which has been associated with susceptibility to infection is the inability to secrete the water soluble glycoprotein forms of the ABO blood group antigens. The prevalence of non-secretors is increased among patients with cholera,[11] urinary tract infection caused by *E coli*,[13] candidiasis,[16] meningococcal meningitis,[17] and pneumococcal infections,[17] but not among those with tuberculosis,[16] leprosy,[16] or gonorrhoea.[12] Though only a few studies have been reported, a pattern is emerging of an association between non-secretion of blood group antigens and susceptibility to infections of the mucosal surfaces.

These observations led us to examine the hypothesis that there would be an increased prevalence of blood group B or non-secretor state, or both, among patients with ankylosing spondylitis and other spondyloarthropathies. As concentrations of IgA (the immunoglobulin associated with protection of mucosal surfaces) are raised in ankylosing spondylitis,[18,19] and reportedly influenced by secretor state,[20,21] we have also determined IgA concentrations in the serum and saliva of our patients.

Patients and methods

We studied 119 patients with clinically well defined spondylarthritides selected at random from the population attending outpatient clinics at the rheumatic diseases unit of the Northern General Hospital over two years. Patients were classified into four categories according to their medical history: ankylosing spondylitis (87 patients), reactive arthritis (22), and radiological sacroiliitis (with or without ankylosing spondylitis) (*a*) associated with inflammatory bowel disease (three patients) or (*b*) associated with psoriasis (seven patients). Patients with ankylosing spondylitis fulfilled the New York criteria,[22] and those with reactive arthritis fulfilled criteria for definite Reiter's disease[23] (14 patients) or postinfective reactive arthritis[24] (eight patients).

When possible patients' records were analysed for HLA state (55 patients) and for a history of ocular inflammation (101) or inflammatory arthritis of peripheral joints (100). A single sample of venous blood and saliva was collected from each of 113 patients. A further five patients provided blood but no saliva, and one provided saliva only.

ABO Blood group was determined in 118 patients by agglutination of red cells with appropriate antisera. Secretor state was determined in 114 patients by the haemagglutination inhibition assay using saliva, as described.[25] Controls were 334 randomly selected normal people attending the Edinburgh blood donor centre during 1981.[13] IgA Concentrations in samples of serum (98 patients) and saliva (97) which had been stored at −20°C were determined by single radial immunodiffusion with commercially prepared plates (Behring Diagnostic, Hounslow).

Statistical analyses were by χ^2 test, using Yates's correction for continuity in fourfold tables. Relative risk (odds ratio) was calculated by simple proportion. Ninety five per cent confidence limits for relative risk were calculated using natural logarithm conversion.

Department of Bacteriology, University of Edinburgh Medical School, Edinburgh EH8 9AG

RUTH SHINEBAUM, BSC, PHD, research fellow
C CAROLINE BLACKWELL, BS, PHD, lecturer
DONALD M WEIR, MD, FRCPED, professor of microbial immunology

Rheumatic Diseases Unit, University of Edinburgh, Northern General Hospital, Edinburgh EH5 2DQ

PETER J G FORSTER, MD, MRCP, senior registrar
NIGEL P HURST, PHD, MRCP, senior registrar
GEORGE NUKI, MB, FRCP, professor of rheumatology

Correspondence to: Dr Shinebaum.

BMJ 146/87

Results

Tables I and II show the distribution of ABO blood group and secretor state in the patients and controls. There was a significant increase ($p<0·001$) in the proportion of non-secretors in the total patient group (54/114; 47%) compared with the local control population (89/334; 27%). The relative risk for spondyloarthropathy in non-secretors was 2·5 (95% confidence interval 1·6–3·8). The increase in non-secretors was evident in all subgroups of patients and achieved statistical significance ($p<0·001$) in both the total group with sacroiliitis (46/97; 47%)—that is, all patients with radiological sacroiliitis irrespective of disease category—and the group with ankylosing spondylitis (41/84; 49%). Nine out of 20 patients with reactive arthritis (45%) and three out of seven with sacroiliitis associated with psoriasis (43%) were non-secretors, proportions similar to that in the group with ankylosing spondylitis.

There was no evidence that the distribution of ABO blood group among the patients was different from that among local controls ($p>0·10$), and in particular no evidence of an increased prevalence of blood group B. The blood group distribution was similar in each disease category.

Analysis of IgA concentrations and clinical features in the total patient group showed few differences between secretors and non-secretors. There was no evidence of differences in serum or salivary IgA concentrations between the two groups (table III); eye lesions were more common among non-secretors (17/48; 35%) than secretors (11/53; 21%), but not significantly so; peripheral joint disease was less common among non-secretors (24/47; 51%) than secretors (35/53; 66%), but again the difference was not significant.

Data on HLA typing were available for 55 patients; 50 were B27 positive and five B27 negative. Three of the B27 negative patients were non-secretors and two were secretors.

TABLE I—*Distribution of ABO blood group among patients with spondyloarthropathies*

	No tested	No (%) with blood group				χ^2 v control	p
		O	A	B	AB		
Control population	334	173 (52)	104 (31)	42 (13)	15 (4)		
Total patient group	118	49 (42)	47 (40)	17 (14)	5 (4)	3·93	>0·10
Total group with sacroiliitis*	99	41 (41)	39 (39)	14 (14)	5 (5)	3·44	>0·10
Ankylosing spondylitis	86	35 (41)	34 (40)	13 (15)	4 (5)	3·51	>0·10
Reactive arthritis	22	10 (45)	8 (36)	4 (18)	0	N/A	
Sacroiliitis associated with inflammatory bowel disease	3	1 (33)	2 (67)	0	0	N/A	
Sacroiliitis associated with psoriasis	7	3 (43)	3 (43)	0	1 (14)	N/A	

N/A = Not applicable.
*All patients with radiological sacroiliitis irrespective of disease category.

TABLE II—*Secretor state of patients with spondyloarthropathies*

	No tested	No (%) of non-secretors	χ^2 v control	p	Relative risk (95% confidence interval)
Control population	334	89 (27)			
Total patient group	114	54 (47)	15·85	<0·001	2·5 (1·6–3·8)
Total group with sacroiliitis*	97	46 (47)	14·13	<0·001	2·5 (1·5–4·2)
Ankylosing spondylitis	84	41 (49)	14·37	<0·001	2·6 (1·5–4·6)
Reactive arthritis	20	9 (45)	2·32	>0·10	2·3 (0·7–7·0)
Sacroiliitis associated with inflammatory bowel disease	3	1 (33)	N/A		
Sacroiliitis associated with psoriasis	7	3 (43)	N/A		

N/A = Not applicable.
*All patients with radiological sacroiliitis irrespective of disease category.

TABLE III—*Secretor state and geometric mean IgA concentrations (SD in parentheses) in patients with spondyloarthropathies. Values are IU/ml*

	Serum IgA	Salivary IgA
Secretors (n=51 serum, 50 salivary)	176·8 (1·5)	4·3 (1·8)
Non-secretors (n=47 serum, 47 salivary)	172·6 (1·9)	4·3 (1·7)

Discussion

Secretion of blood group antigens into body fluids is a dominant, genetically determined characteristic. Roughly 25% of people throughout the world are recessive for the secretor (*Se*) gene, genotype *se,se*, though the proportion varies in some populations.[5] Our control group shows that the local population had a proportion of non-secretors similar to but slightly higher than the expected frequency, as is usual among populations of Celtic origin.

This study found a significantly increased prevalence of non-secretors of blood group antigens among patients with spondylo-arthropathies. There are several possible explanations. The gene for secretion may be linked to another which affects susceptibility to disease or immune responsiveness; the product of the *Se* gene may modify antigens on cells of host or parasite; or blood group antigens may themselves be important in determining susceptibility to these diseases.

Family studies of ankylosing spondylitis and reactive arthritis show that genetic determinants additional to HLA-B27 are necessary for the development of these diseases.[7,8] It has been suggested that such additional genes are concerned with the functioning of the immune system, and it is therefore tempting to speculate that there is a genetic link between secretor state and immune responsiveness. There is evidence that the *Se* gene forms part of a linkage group on chromosome 19 with complement component C3, but this is the only known immunological function coded for nearby: genes coding for HLA and most other immune determinants are on chromosome 6.[9] A single report that salivary IgA concentrations are reduced in non-secretor adults[10] suggests that IgA deficiency may be responsible for increased susceptibility to infection in non-secretors, which in turn might predispose to reactive arthritis and spondylarthritis. Our data do not support such a hypothesis. Our studies suggest that non-secretion increases the relative risk for ankylosing spondylitis and reactive arthritis, albeit only twofold to threefold. Hence the gene determining secretor state is possibly in itself a subsidiary determinant for these diseases. Family studies will be required to assess the combined relative risk of being HLA-B27 positive and the non-secretor genotype *se,se*.

The biological role of the *Se* gene is not clear, but there is

biochemical evidence that *Se* is a structural gene encoding a specific fucosyltransferase. At least two such enzymes are concerned with the synthesis of blood group specificities, adding fucose residues to one of several types of precursor chain. Evidence suggests that the *Se* gene product preferentially uses the type I chain as substrate.[28] Possibly this enzyme glycosylates other structures such as HLA or other cell surface antigens, or even antigens on invading micro-organisms. Any such modification of antigens on host tissue or infecting agent might affect the balance of the initial host-parasite interaction.

The main differences between secretors and non-secretors are related to the antigens present in their body fluids. With the exception of the extremely rare Bombay phenotype, all secretors have the water soluble form of the H antigen (the antigen of blood group O), and the 94% who possess the Lewis blood group gene have Le^a antigen. People of the appropriate blood groups will also secrete water soluble forms of the A and B antigens. Non-secretors do not have A, B, H, or Le^a antigens in their body fluids, but the 94% with the Lewis gene have a different form of the Lewis antigen, Le^b. ABH antigens occur in two forms, a glycolipid which forms part of the structure of a cell and a glycoprotein which is found in body fluids. Lewis antigens occur as glycolipids in the plasma and as glycoproteins in secretions; they do not form part of the structure of cells but are passively adsorbed to cell surfaces.

Much epidemiological evidence suggests that ankylosing spondylitis and reactive arthritis occur in genetically predisposed subjects after exposure to micro-organisms. Finding an association between secretor state and spondyloarthropathies suggests a possible role for blood group antigens in these diseases. Non-secretion appears to be a marker associated with infections of the mucosal surfaces[13 15 16] and may be important in the colonisation stages of an infectious process. We suggest two models to explain the increased susceptibility of non-secretors to initial infection: Le^a in the body fluids of non-secretors may act as a receptor for infecting micro-organisms; or H or Le^a, or both, in the body fluids of secretors may inhibit the attachment of organisms. These two mechanisms are not mutually exclusive. It is unlikely that water soluble forms of the A and B antigens play a part in the spondyloarthropathies, as our studies showed no association between these blood groups and ankylosing spondylitis or reactive arthritis.

There is at present little direct evidence that the ABO or Lewis blood groups act as receptors for micro-organisms. Nevertheless, antigens of other blood group systems do act in this way,[11 12] and recent work from our laboratory has identified the Le^a antigen as a receptor for certain strains of *Candida*.[11] Indirect evidence for a receptor role for these antigens comes from finding that *E coli* binds in greater numbers to non-secretor than secretor cells,[34] and from studies showing that attachment of micro-organisms may be inhibited by glycocompounds in body secretions.[35 36] Experiments are under way in our laboratory to see whether any of these mechanisms are operative in the interaction of arthritogenic Gram negative bacteria with secretor and non-secretor cells of patients with ankylosing spondylitis and controls.

Finding that non-secretion is associated with ankylosing spondylitis and reactive arthritis, together with the accumulating evidence of an association between non-secretion and mucosal surface infections, strengthens the hypothesis that ankylosing spondylitis is a form of reactive arthritis.

Though the increased relative risk for spondylarthritis associated with non-secretion is less than that associated with HLA-B27, non-secretion is nevertheless a host susceptibility factor for these diseases. Moreover, it may be particularly important in pointing the way to our understanding of the pathogenetic mechanisms in the initiation of disease in susceptible people.

This work was supported by a project grant from the Arthritis and Rheumatism Council. We thank the Edinburgh and South East Scotland Blood Transfusion Service for essential reagents and Dr R Prescott, of the University of Edinburgh, for statistical advice. We also thank Mrs M Cole for help in preparing the manuscript.

References

1 Ebringer A. The link between genes and disease. *New Scientist* 1978;79:865-7.
2 Kassella TD, Frizzier MJ, McNeil DJ. Ankylosing spondylitis. A disease in search of microbes. *J Rheumatol* 1983;10:2-4.
3 Neumann V, Wright V. Arthritis associated with bowel disease. *Clin Gastroenterol* 1983;12: 767-95.
4 Kind CRK. Reactive arthritis. *Postgrad Med J* 1982;58:131-7.
5 Ebringer R, Cooke D, Crowell DR, Cowling P, Ebringer A. Ankylosing spondylitis: Klebsiella and HL-A B27. *Rheumatol Rehabil* 1977;16:190-6.
6 Eastmond CJ, Willshaw HE, Burgess SEP, Shinebourn R, Cooke EM, Wright V. Frequency of faecal Klebsiella serogroups in patients with ankylosing spondylitis and controls with respect to individual features of the disease. *Ann Rheum Dis* 1980;39:118-23.
7 Warren RE, Brewerton DA. Faecal carriage of klebsiella by patients with ankylosing spondylitis and rheumatoid arthritis. *Ann Rheum Dis* 1980;39:37-44.
8 Geczy AF, Yap J. A survey of isolates of Klebsiella pneumoniae which cross-react with an HLA-B27-associated cell-surface structure on the lymphocytes of patients with ankylosing spondylitis. *J Rheumatol* 1982;9:97-100.
9 Bessiere A, Rousseau F, Israel-Assayag E, Roy R. Klebsiella related antigens in ankylosing spondylitis. *J Rheumatol* 1983;10:102-5.
10 Mourant AE, Kopec AC, Domaniewska-Sobczak K. *Blood groups and disease: a study of associations of diseases with blood groups and other polymorphisms*. Oxford: Oxford University Press, 1978.
11 Barua D, Pegues AS. ABO blood groups and cholera. *Ann Hum Biol* 1977;4:489-92.
12 Kinane DF, Blackwell CC, Winstanley FP, Weir DM. Blood group, secretor status, and susceptibility to infection by Neisseria gonorrhoeae. *Br J Vener Dis* 1983;59:44-6.
13 Kinane DF, Blackwell CC, Brettle RP, Weir DM, Winstanley FP, Elton RA. ABO blood group, secretor state, and susceptibility to recurrent urinary tract infection in women. *Br Med J* 1982;285:7-9.
14 Robinson MG, Tolchin D, Halpern C. Enteric bacterial agents and the ABO blood groups. *Am J Hum Genet* 1971;23:135-45.
15 Chaudhuri A, Das Adhikary CR. Possible role of blood-group secretory substances in the aetiology of cholera. *Trans R Soc Trop Med Hyg* 1978;72:664-5.
16 Blackwell CC, Thom SM, Weir DM, Kinane DF, Johnstone FD. Host-parasite interactions underlying non-secretion of blood group antigens and susceptibility to infections by Candida albicans. In: Lark DL, ed. *Protein-carbohydrate interactions in biological systems*. London: Academic Press, 1986:231-5.
17 Blackwell CC, Jonsdottir K, Hanson M, *et al*. Non-secretion of ABO antigens predisposing to infection by Neisseria meningitidis and Streptococcus pneumoniae. *Lancet* 1986;ii:284-5.
18 Veys EM, Van Laere M. Serum IgG, IgM and IgA levels in ankylosing spondylitis. *Ann Rheum Dis* 1973;32:493-6.
19 Laurent MR, Panayi GS. Acute-phase proteins and serum immunoglobulins in ankylosing spondylitis. *Ann Rheum Dis* 1983;42:524-8.
20 Gruadbacher FJ. Immunoglobulins, secretor status and the incidence of rheumatic fever and rheumatic heart disease. *Hum Hered* 1972;25:399-404.
21 Wassolitch JG, Langman MJS. ABO blood groups, secretor status, salivary protein, and serum and salivary immunoglobulin concentrations. *Gut* 1971;12:646-9.
22 Bennett PH, Burch TA. New York symposium on population studies in the rheumatic diseases: new diagnostic criteria. *Bull Rheum Dis* 1967;17:453-8.
23 Wilkens RF, Arnett FC, Bitter T, *et al*. Reiter's syndrome. Evaluation of preliminary criteria for definite disease. *Arthritis Rheum* 1981;24:844-9.
24 Ohlages B. Postinfective or reactive arthritis. *Scand J Rheumatol* 1980;9:193-202.
25 Mollison PL. *Blood transfusion in clinical medicine*. Oxford: Blackwell Scientific Publications, 1979:414-82.
26 Mourant AE, Kopec AC, Domaniewska-Sobczak K. *The distribution of the human blood groups and other polymorphisms*. London: Oxford University Press, 1976
27 Woodrow JC. Genetic analysis of ankylosing spondylitis. In: Ziff M, Cohen SB, eds. *The spondyloarthropathies. Advances in inflammation research*. Vol 9. New York: Raven Press, 1985:75-82.
28 Brewerton DA. HLA-B27 and the significance of susceptibility to rheumatic disease. *Arthritis Rheum* 1976;19:656-68.
29 McKusick VA. The human gene map. In: O'Brien SJ, ed. *Genetic maps*. Vol 2. New York: Cold Spring Harbor Laboratory, 1983:327-49.
30 Kumazaki T, Yoshida A. Biochemical evidence that secretor gene, Se, is a structural gene encoding a specific fucosyltransferase. *Proc Natl Acad Sci USA* 1984;81:4193-7.
31 Kallenius G, Mollby R, Svenson SB, *et al*. The pk antigen as receptor for the haemagglutinin of pyelonephritic Escherichia coli. *FEMS Microbiological Letters* 1980;7:297-302.
32 Miller LH, Mason SJ, Dvorak JA, McGinniss MH, Rothman IK. Erythrocyte receptors for (Plasmodium knowlesi) malaria: Duffy blood group determinants. *Science (New York)* 1975;189:561-3.
33 May SJ, Blackwell CC, Weir DM. Non-secretion of blood group antigens and susceptibility to Candida albicans: the role of Lewis blood group antigens [Abstract]. *J Dent Res* 1986;65:503.
34 Lomberg H, Cedergren B, Leffler H, Nilsson B, Carlstrom A-S, Svanborg-Eden C. Influence of blood group on the availability of receptors for attachment of uropathogenic Escherichia coli. *Infect Immun* 1986;51:919-26.
35 Williams RC, Gibbons RJ. Inhibition of streptococcal attachment to receptors on human buccal epithelial cells by antigenically similar salivary glycoproteins. *Infect Immun* 1975;11:711-8.
36 Holmgren J, Svennerholm A-M, Ahren C. Non-immunoglobulin fraction of human milk inhibits bacterial adhesion (hemagglutination) and enterotoxin binding of Escherichia coli and Vibrio cholerae. *Infect Immun* 1981;33:136-41.

(Accepted 18 November 1986)

Printed in Great Britain by Bourne Offset Ltd., Iver, Bucks.

J. Clin. Lab. Immunol. (1987), 24, 29–32

LIPOPOLYSACCHARIDE STRUCTURE AND SERUM SENSITIVITY OF NON-SEROGROUPABLE *NEISSERIA MENINGITIDIS*

C. C. BLACKWELL, F. P. WINSTANLEY, D. M. WEIR and D. F. KINANE
Department of Bacteriology, University of Edinburgh, Medical School, Teviot Place, Edinburgh EH8 9AG, UK
(Received 11 March 1987)

SUMMARY Bactericidal activities of normal human serum for non-serogroupable strains of *Neisseria meningitidis* were determined. In similar experiments with isolates of *Neisseria gonorrhoeae* from localized infections, strains with group I lipopolysaccharide (LPS) were uniformly serum resistant and those with group II were serum sensitive. We found no similar association between serum sensitivity of the meningococcal strains and their lipopolysaccharide groups determined by the same pyocin typing system used to classify the gonococcal isolates.

Immune mouse sera raised against non-serogroupable meningococci of either LPS group I or II were bactericidal for non-serogroupable strains of the same LPS group and also cross-reactive for strains of the opposite group. They were not bactericidal for the majority (13/17) of the serogroupable strains tested.

These findings suggest there are antigens, in addition to the LPS and capsules, that elicit some of the "natural" bactericidal antibodies to pathogenic meningococci.

Key words: Serum sensitivity, meningococci, lipopolysaccharide, natural antibodies

INTRODUCTION

IN studies on host-parasite interactions influencing gonococcal infection, killing of *N. gonorrhoeae* by normal human serum depended on the lipopolysaccharide (LPS) structure of the bacteria. A pyocin typing scheme divided strains into 2 major groups. Group I strains, those with a "simple" LPS, were inhibited only by pyocins of the R5 class as defined by Kageyama (1); group II strains, those with a "complex" LPS, were inhibited by pyocins of the R5 class and by pyocins of the R1 class. The group I strains were uniformly resistant to the bactericidal activity of normal human serum while those of group II were serum-sensitive. Immune mouse sera raised against either group I or group II organisms were bactericidal for other strains of the same group and also cross-reactive for strains of the opposite group (2).

LPS is the target antigen for "natural" bactericidal antibodies against gonococci in normal human serum (NHS) and a correlation between the presence of bactericidal antibodies and immunity to meningococcal disease has long been established (3). Development of antibodies to pathogenic strains of meningococci has been associated with carriage of the usually non-pathogenic, non-serogroupable strains of the organism (4, 5). Previously we divided non-serogroupable strains of *N. meningitidis* into the same 2 groups as the gonococci with the pyocin typing system (6). In this study sera from the same population tested for bactericidal activity to gonococci were screened to determine if there was a similar correlation between LPS structure and susceptibility to "natural" antibodies for non-serogroupable meningococci.

The bactericidal activity of antisera produced by mice inoculated with non-serogroupable meningococci of group I or group II was examined to test the hypothesis that these might be responsible for induction of bactericidal antibodies to serogroupable strains.

Correspondence: Dr. C. C. Blackwell, Department of Bacteriology, University of Edinburgh, Medical School, Teviot Place, Edinburgh EH8 9AG, UK.

MATERIALS AND METHODS

Bacterial Strains

Non-serogroupable strains of *N. meningitidis* were obtained from Dr. J. Etienne and Professor J.-P. Albert, Centre Collabrateur, OMS de Reference et de Recherche pour les Meningococques, Marseille-Armees, France and Dr. R. J. Fallon, Ruchill Hospital, Glasgow: 5 group I strains, 6 group II strains and 6 strains resistant to all the pyocins in the typing scheme. Those strains with PA before the number were polyag-

0141-2760/87/0900-0029 $03.50

by any [illegible] standard typing sera. Representatives of each of the following serogroups were also included: (number in brackets is the number of strains tested); A (1), B (6), C (3), D (1), W135 (4), Y (1), Z' (2). Each conformed to the morphological and biochemical characteristics of *N. meningitidis*.

Culture

Strains were cultured on Modified New York City (MNYC) medium (7) at 37°C in a humidified atmosphere enriched with 10% CO_2.

Pyocin Typing

Strains of non-serogroupable meningococci were divided into group I, group II or pyocin resistant categories by the pyocin typing method described previously (6, 8).

Preparation of Immunizing Antigens and Immunization Schedule

Immune mouse sera to isolates of group I (42619-4) or group II (1419) were produced as described for gonococcal strains (2).

Complement

Human serum from a blood group AB donor with no history of gonococcal infection was absorbed at 4°C with a live suspension of a "cocktail" of the serogroupable strains and the non-serogroupable strains of group I or group II used to immunize the mice. After 4 hours the bacteria were removed by centrifugation and the serum was absorbed a second time overnight. The absorbed serum was distributed in 1 ml aliquots and stored at −20°C before use. It contained no bactericidal activity for the strains tested and the minimum haemolytic titre of the serum was 32–64.

Normal Human Serum from Women of Blood Groups A and B

These were obtained from women attending post-natal clinics and kindly supplied by Dr. S. Urbaniak and Dr. P. L. Yap of the Blood Transfusion Service, Royal Infirmary, Edinburgh (RIE). They were heated to 56°C for 30 min and stored at −20°C.

serum dilution to give a reduction in viable count ⩾80% compared with the controls was taken as the bactericidal titre of the serum. The small quantities of human sera available allowed only one titration to be made against each strain. Control positive sera were included for each test to ensure the experimental system was functioning correctly. The mouse sera were tested 3 times.

Bactericidal Assay with Immune Mouse Serum

These were performed as described previously with heat inactivated immune mouse serum and freshly-thawed, absorbed human AB serum as the complement source (2). A reduction in viable count ⩾ 80% compared with controls was considered to be significant bactericidal activity.

Results

"Natural" Human Antibodies

"Normal human sera" from 48 women of blood groups A and B attending a postnatal clinic were screened for bactericidal activity against meningococci. This population had a very low incidence of gonorrhoea and was used in the previous study to determine the presence of "natural" bactericidal antibodies to *N. gonorrhoeae*. Results for non-serogroupable and serogroupable isolates are shown in Table 1. There was no clear cut pattern such as that observed for gonococci. Regardless of LPS type, bactericidal activity was observed for each of the non-serogroupable strains, serogroups A and W135, but none was found against Y. The distributions of titres for sera of blood groups A and B against each serum

Table 1 Frequency distribution of titres of normal human serum bactericidal activity against strains of *N. meningitidis*

		No. sera with bactericidal activity (log titre)							
	No. sera	*< 0·6*	*0·6*	*0·9*	*1·2*	*1·5*	*1·8*	*2·1*	*2·6*
Non-serogroupable strains									
LPS group I									
PA 1190	36	5	7	1	1	7	4	6	5
NA 935113	31	2	15	0	0	2	4	4	4
42619-4	16	0	5	0	1	0	3	1	6
PA 477	33	4	19	1	1	1	2	3	2
LPS group II									
1419	32	3	5	1	0	4	3	10	6
PA 469	30	5	5	0	0	0	1	9	10
0452	25	0	0	0	1	0	0	18	6
14795	30	4	2	4	11	5	3	1	0
Pyocin resistant									
PA 846	32	9	3	4	3	4	9	0	0
PA 520	31	0	9	0	1	0	0	0	21
Serogroupable strains									
ref A	29	8	1	3	1	8	4	3	1
ref Y	32	32	0	0	0	0	0	0	0
W 135	16	6	1	4	3	2	0	0	0

Table 2 Sensitivity of non-serogroupable isolates of *N. meningitidis* to mouse antisera raised against non-serogroupable meningococci with group I or group II LPS

	LPS group of immunizing strain	
Isolate	*I*	*II*
Group I		
*42619-4	+	+
NA 935113	+	+
PA 1190	0	+
1790	+	+
Group II		
*1419	+	+
16795	+	+
NA 0352	0	0
PA 469	+	+
795	0	0
0452	+	+
Nontypable		
PA 846	+	0
PA 520	+	+
NA 499	+	+
NA 879	+	+
76/15373	0	0
NA 1784	0	0

+ = > 80% decrease in viable count of control.
0 = no significant bactericidal activity.
* strain used as immunogen.

sensitive strain were compared by the Mann-Whitney U-test. No significant differences were observed in a one-tailed test at a probability level of 0·025.

Immune Mouse Antibodies

Mouse antisera raised against non-serogroupable strains of either group I or II meningococci were bactericidal for a number of non-groupable strains (Table 2). The majority of serogroupable strains (13/17) were not killed by either mouse serum. Each of the strains of serogroups A, B, C and D were resistant to both antisera. Both 29E strains were sensitive to these antisera as were 2 of the 4 W135 strains.

Discussion

Schneider and co-workers (9) reported that resistance of gonococci to NHS was due to the lack of an LPS determinant that binds "natural" antibody. Our previous study suggested that among isolates from localized infections this antigen is found on serum sensitive strains with a "complex" LPS (group II) but not on those serum resistant ones with a "simple" LPS (group I) (2). We also found that induction of phenotypic serum resistance was correlated with a change from group II to group I LPS (10). This alteration in LPS structure has since been confirmed (11). We did not observe this pattern of serum sensitivity among non-serogroupable strains of meningococci divided into group I and group II by the same pyocin typing method used for gonococci.

Experiments with mouse antisera suggest that bactericidal antibodies can be induced by non-serogroupable strains of meningococci of LPS group I or group II. As with the mouse antisera produced against group I and group II gonococci (2), cross reactions among strains from both LPS groups were found (Table 2). Since the LPS is one of the principal target antigens for bactericidal antibody, it was predicted that specific antibodies produced against non-serogroupable strains might be effective against serogroupable capsulate ones. The results obtained are evidence against this hypothesis; there was no bactericidal activity against strains of serogroups A, B, C or D. Both strains of group 29E were sensitive to these sera. These results are similar to those reported by Craven *et al.* (12), each of their four 29E strains were serum sensitive. The sensitivity of the two W135 strains might be due to a decrease of capsular material following subculture resulting in the underlying LPS being more accessible to the antibodies. Pyocin typing data suggests that the LPS of non-serogroupable strains is more accessible; 66% of non-serogroupable strains tested were sensitive to pyocins compared to only 11% of serogroupable strains (6). In contrast, other workers (12) were unable to correlate serum sensitivity with quantities of extractable group B polysaccharide.

These findings suggest, as do those of Craven *et al.* (12) that induction of "natural" bactericidal antibodies to pathogenic meningococci is dependent on the host's response to more than one group of antigens. Additional evidence for this comes from one of our earlier studies. It had been predicted that since the LPS of gonococci and meningococci are structurally similar (13), gonococcal infection might act as a stimulus for bactericidal antibodies against *N. meningitidis*. Sera from patients with meningococcal infection have been found to have increased levels of bactericidal antibodies to serum sensitive gonococci (14). There were however decreases in the titre of antibodies in the sera of patients with gonorrhoea against serogroups A, B and C but not against non-serogroupable strains (15).

These findings suggest the presence of antigens, in addition to those of the LPS and capsules, common to both gonococci and meningococci, that are responsible for induction of some of the "natural" bactericidal antibodies that protect the host from meningococcal disease.

ACKNOWLEDGEMENTS

We are most grateful to Mrs. M. Cole for typing the manuscript. The work was supported by grants from the Medical Research Council (G81/103811/SB and G979/64/S). Dr. D. F. Kinane was a recipient of the Davidson Research Fellowship, Faculty of Medicine, University of Edinburgh.

REFERENCES

1. Kageyama, M. (1975). Bacteriocins and bacteriophages in *Pseudomonas aeruginosa*. In *Microbial Drug Resistance* (edited by S. Mitsuhashi and H. Hashimoto), pp. 291–305. University Park Press, Baltimore.
2. Winstanley, F. P., Blackwell, C. C., Weir, D. M. and Kinane, D. F. (1983). ABO blood groups and susceptibility to gonorrhoea. II. The relationship of lipopolysaccharide type to gonococcal sensitivity to the bactericidal activity of normal human serum. *J. Clin. Lab. Immunol.*, **11**, 27–32.
3. Goldschneider, I., Gotschlich, E. C. and Artenstein, M. S. (1969a). Human immunity to the meningococcus I. The role of humoral antibodies. *J. Exp. Med.*, **129**, 1307–1326.
4. Goldschneider, I., Gotschlich, E. C. and Artenstein, M. S. (1969b). Human immunity to the meningococcus II. Development and natural immunity. *J. Exp. Med.*, **129**, 1327–1348.
5. Reller, L. B., MacGregor, R. B. and Beatty, H. N. (1973). Bactericidal antibody after colonization with *Neisseria meningitidis*. *J. Infect. Dis.*, **127**, 56–62.
6. Blackwell, C. C. and Law, J. A. (1981). Typing of non-serogroupable *Neisseria meningitidis* by means of sensitivity to R-type pyocins of *Pseudomonas aeruginosa*. *J. Infect.*, **3**, 370–378.
7. Young, H. (1978). Cultural diagnosis of gonorrhoea with modified New York City (MNYC) medium. *Br. J. Vener. Dis.*, **54**, 36–40.
8. Blackwell, C. C., Young, H. and Anderson, I. (1978). Sensitivity of *Neisseria gonorrhoeae* to partially purified R-type pyocins and a possible approach to epidemiological typing. *J. Med. Microbiol.*, **12**, 321–335.
9. Schneider, H., Griffis, J. M., Williams, G. D. and Pier, G. B. (1982). Immunological basis of serum resistance of *Neisseria gonorrhoeae*. *J. Gen. Microbiol.*, **128**, 13–22.
10. Winstanley, F. P., Blackwell, C. C., Tan, E. L., Patel, P. V., Parsons, N. J., Martin, P. M. V. and Smith, H. (1984). Alteration of pyocin-sensitivity pattern of *Neisseria gonorrhoeae* is associated with induced resistance to killing by human serum. *J. Gen. Microbiol.*, **130**, 1303–1306.
11. Tan, E. L., Patel, P. V., Parsons, N. J., Martin, P. M. V. and Smith, H. (1986). Lipopolysaccharide alteration is associated with induced serum resistance of *Neisseria gonorrhoeae* and killing by human serum. *J. Gen. Microbiol.*, **132**, 1407–1413.
12. Craven, D. E., Shen, K. T. and Frasch, C. E. (1982). Natural bactericidal activity of human serum against *Neisseria meningitidis* isolates of different serogroups and serotypes. *Infect. Immun.*, **37**, 132–137.
13. Tramont, E. C., Sadoff, J. C. and Artenstein, M. S. (1974). Cross-reactivity of *Neisseria gonorrhoeae* and *Neisseria meningitidis* and the nature of antigens involved in the bactericidal reaction. *J. Infect. Dis.*, **130**, 240–247.
14. Cremieux, A. C., Puissant, A., Ancelle, R. and Martin, P. M. V. (1984). Bactericidal antibodies against *Neisseria gonorrhoeae* elicited by *Neisseria meningitidis*. *Lancet*, **ii**, 930.
15. Winstanley, F. P., Blackwell, C. C., Weir, D. M. and Kinane, D. F. (1983). Gonorrhoea: a predisposing factor for meningococcal disease? *Lancet*, **ii**, 1134–1135.

Teviot-Kimpton Publications
2-3 Teviot Place
Edinburgh EH1 2QZ, UK

This study has shown that amitriptyline and several related tricyclic antidepressants have higher fatal toxicity indices than the monoamine oxidase inhibitors, which are in turn more toxic than several newer antidepressants. All of the drugs introduced since 1973 have a favourable toxicity profile except maprotiline. If the newer drugs have as good a record of clinical effectiveness, combined with their apparent lower potential to cause fatal poisoning when taken in overdose, serious consideration should be given to preferentially prescribing the newer drugs, especially to patients who are considered at particular risk of suicide by ingestion of an overdose of their medication.

We thank Squibb Europe Inc, Roussel Laboratories Ltd, Smith Kline and French Ltd, Pfizer Ltd, and the Lawson Tait (Humane Research) Trust, for financial support. We thank the statistics and research division of the Department of Health and Social Security for giving us access to prescription data and the Office of Population Censuses and Surveys for providing us with unpublished mortality data. We also thank Ms J Turner for statistical advice and H Wiseman for help in preparing the manuscript.

References

1 Brewer C, Farmer R. Self poisoning in 1984: a prediction that didn't come true. *Br Med J* 1985;290:391.
2 Ouseltos MD, Blackmore RC, King LA, Moffat AC. Poisoning associated deaths between 1973 and 1980. *Hum Toxicol* 1984;3:201-21.
3 Barraclough BM. Are there safer hypnotics than barbiturates? *Lancet* 1974;i:57-8.
4 Girdwood RH. Death after taking medicaments. *Br Med J* 1974;ii:501-4.
5 King LA, Moffat AC. A possible index of fatal drug toxicity in humans. *Med Sci Law* 1983;23:193-8.
6 Stead AH, Moffat AC, King LA. Quantitative structure-activity relationships in forensic toxicology. In: Dearden JC, ed. *Quantitative approaches to drug design*. Oxford: Elsevier, 1983:277-8.
7 British Medical Association and the Pharmaceutical Society of Great Britain. *British national formulary*. London: British Medical Association, 1975-84.
8 Associations of the British Pharmaceutical Industry. *Data sheet compendium*. London: Associations of the British Pharmaceutical Industry, 1975-84.
9 Office of Population Censuses and Surveys. *Mortality statistics—accidents and violence (England and Wales)*. London: HMSO, 1975-84. (Series DH4.)
10 Registrar General. *Annual report: mortality statistics*. Scotland: General Register Office, 1975-84.
11 Blackwell B. Antidepressant drugs. In: Dukes MNG, ed. *Meyler's side effects of drugs*. 10th ed. Oxford: Elsevier, 1984:34-61.
12 Vale JA, Buckley BM, Meredith TJ. Deaths from paracetamol and dextropropoxyphene (Distalgesic) poisoning in England and Wales in 1979. *Hum Toxicol* 1984;3(suppl):135-43S.
13 Callaham M, Kassel D. Epidemiology of fatal tricyclic antidepressant ingestion: implications for management. *Ann Emerg Med* 1985;14:1-9.
14 Crome P, Newman B. Why people die from tricyclic antidepressant poisoning. *Vet Hum Toxicol* 1979;21(suppl):56-8.
15 Crome P, Newman B. The problem of tricyclic antidepressant poisoning. *Postgrad Med J* 1979;55:528-32.
16 Prasad PR, Benowitz NL. Tricyclic antidepressant poisoning: management of arrhythmias. *Medical Toxicology* 1986;1:161-21.
17 King LA. Ferguson's principle and the prediction of fatal drug levels in blood. *Hum Toxicol* 1985;4:273-8.
18 Wedin GP, Odeida GM, Klein-Schwartz W, Gorman RL. Relative toxicity of cyclic antidepressants. *Ann Emerg Med* 1986;15:797-804.
19 Nicholander RC, Zimmerman J, Hynes MD, Steinberg MI, Sullivan HR. Pharmacologic and toxicologic effects in animals of dextropropoxyphene and its major metabolite norpropoxyphene: a review. *Hum Toxicol* 1984;3(suppl):13-36S.
20 Zaman S, Lamb JM, Esberger DA, Pearson RM. Inhibition of sperm motility by opiate drugs. *Br J Clin Pharmacol* 1984;18:382P.
21 Yasuhara H, Tanooka M, Kanai K, Sakamoto K. Membrane effects of various drugs on isolated rat hepatocytes and erythrocytes. *Toxicol Appl Pharmacol* 1985;79:453-60.
22 Yasuhara H, Matano H, Sakamoto K, Ueda I. Mechanism of membrane stabilising and lytic effects of tricyclic antidepressants. *Jpn J Pharmacol* 1980;30:397-401.
23 Kulig K. Management of poisoning associated with "newer" antidepressant agents. *Ann Emerg Med* 1986;15:1039-44.
24 Wakeling A. Efficacy and side effects of mianserin, a tetracyclic antidepressant. *Postgrad Med J* 1983;59:229-31.
25 Ali CJ, Henry JA. Trazodone overdosage. Experience over five years. *Neuropsychobiology* 1986;15(suppl 1):44-5.
26 Rawls WN. Trazodone. *Drug Intell Clin Pharm* 1982;16:7-13.
27 Cassidy SL, Henry JA. Rapid in vitro techniques for the assessment of the membrane stabilizing activity of drugs. *Food Chem Toxicol* 1986;24:807-9.
28 Leonard BE. Toxicity of antidepressants. *Lancet* 1986;i:1105.

(Accepted 9 September 1987)

SHORT REPORTS

Secretor state of patients with insulin dependent or non-insulin-dependent diabetes mellitus

The inability to secrete the water soluble glycoprotein form of the ABO blood group antigens is associated with increased susceptibility to several infections, particularly among children in the vulnerable period between losing maternal antibodies and developing their own active immunity.[1] This is a characteristic that does not alter with age or environmental influences.

The secretor (*Se*) gene is located on chromosome 19; it is not linked to sex or to the histocompatibility locus antigen (HLA) markers. We have also found a considerable increase in the proportion of non-secretors (*se*) among patients who have ankylosing spondylitis, a rheumatic condition that has a close association (about 95% of cases) with the HLA B27 marker and for which an infectious aetiology has been postulated.[2] As viral infections have been suggested to contribute to the development of type I diabetes,[3] a recent editorial on the genetics of this disorder[4] prompted us to compare the secretor state of patients who have type I disease with those who have type II disease. If there were a parallel with ankylosing spondylitis we predicted that there would be a higher proportion of non-secretors among the patients who had type I diabetes.

Patients, methods, and results

During six weeks 205 diabetic patients were examined (102 insulin dependent (56 men and 46 women); 103 non-insulin-dependent (60 men and 43 women)). The patients were recruited from those attending routine clinics; the first five to 12 patients at each session were asked to participate. None of the patients was admitted to hospital. Patients who had type I disease were classified by insulin dependence, their clinical history, and family history of the disease. The patients who had type II disease were not dependent on insulin.

The ABO and Lewis blood groups were determined by agglutination and the secretor state by the haemagglutination inhibition method[5] from saliva. The Lewis group was used to confirm the results obtained from the saliva sample. The results were compared with those found for local blood donors by χ^2 test.

There was no significant difference in the distribution of the ABO blood groups between the insulin dependent and non-insulin-dependent diabetic patients ($\chi^2=0{\cdot}5911$, df=3, $p>0{\cdot}50$), and the results were similar to those found for local blood donors (type I: $\chi^2=3{\cdot}702$, df=3, $p>0{\cdot}10$; type II: $\chi^2=1{\cdot}266$, df=3, $p>0{\cdot}50$). Among the patients who had type II disease there was no significant difference in the proportion of non-secretors (25%) compared with the controls. Among the patients who had type I disease the proportion of non-secretors was significantly increased (40%) ($\chi^2=6{\cdot}223$, $p<0{\cdot}02$) (table).

Blood group and secretor state of patients with type I and type II diabetes mellitus. All figures are numbers (percentages) of patients

	ABO blood group				Secretor state		
	A	B	O	AB	Secretor (*Se*)	Non-secretor (*se*)	Significance
Diabetic patients:							
Type I (n=102)	35 (34)	11 (11)	56 (55)		61 (60)	41 (40)	p<0·02
Type II (n=103)	37 (36)	11 (11)	53 (51)	2 (2)	77 (75)	26 (25)	NS
Controls (n=334)	104 (31)	42 (13)	173 (52)	15 (4)	245 (73)	89 (27)	

Comment

Among the patients studied there were probably clinical and genetic heterogeneities within the insulin dependent and non-insulin-dependent groups; secretor state, however, is an additional, easily determined genetic

marker that can be used further to investigate differences in the two main aetiological types of diabetes.

The parallel of our results for type I diabetes with those for ankylosing spondylitis is striking. In both there are strong associations with particular HLA markers and a significant increase in the proportion of non-secretors. This is further indirect evidence to support the suggestion that an infectious agent is implicated in the initiation of type I diabetes in some genetically predisposed patients. Determining the secretor state of patients who have various viral infections would be a useful first step in investigating this hypothesis. We suggest that the initial susceptibility to some viral infections might be greater in the non-immune, non-secretor host. The specific immune response to the putative infectious agent, controlled by the class II HLA DR3/4 genes, might contribute to pathogenic sequelae leading to the diabetic state.

We are grateful to Dr A E Mourant for helpful discussions and to the Edinburgh and South East Scotland Blood Transfusion Service for reagents used in the study.

1 Blackwell CC, Jonsdottir K, Hanson M, *et al.* Non-secretion of ABO antigens predisposing to infection by Neisseria meningitidis and Streptococcus pneumoniae. *Lancet* 1986;ii:284-5.
2 Shinebaum R, Blackwell CC, Forster PJG, *et al.* Non-secretion of ABO blood group antigens as a host susceptibility factor in the spondyloarthropathies. *Br Med J* 1987;294:208-10.
3 Bottazo GF. Death of a beta cell: homicide or suicide? *Diabetic Medicine* 1986;3:119-30.
4 Keen H. The genetics of diabetes: from nightmare to headache. *Br Med J* 1987;294:917-9.
5 Mollison PL. *Blood transfusion in clinical medicine.* Oxford: Blackwell Scientific Publications, 1979:414-82.

(Accepted 17 August 1987)

Department of Bacteriology, The Medical School, University of Edinburgh, Edinburgh EH8 9AG
C CAROLINE BLACKWELL, BSC, PHD, lecturer
VALERIE S JAMES, FIMLS, research technician
DONALD M WEIR, MD, FRCPED, professor

Diabetic and Dietetic Department, Royal Infirmary, Edinburgh EH3 9YW
JOHN D GEMMILL, BSC, MRCP, registrar
ALAN W PATRICK, MRCP, registrar
ANDREW COLLIER, BSC, MRCP, registrar
BASIL F CLARKE, FRCPED, consultant physician

Correspondence to: Dr Blackwell.

Quality of haemofiltration fluids: a potential cause of severe electrolyte imbalance

Haemofiltration, which requires the infusion of large amounts of electrolyte replacement solutions, is finding increasing application in general hospitals. These solutions do not have a product licence, so responsibility for their quality, safety, and efficacy is taken by the prescriber. We report a case in which the wide limits of quality control of a solution had clinical implications and discuss a survey of the limits of quality control of some commonly used haemofiltration fluids.

Patient, method, and results

A 73 year old woman was admitted to hospital because of worsening breathlessness and peripheral oedema. She had a history of two mitral valve replacements nine years and two months previously. The clinical findings were severe tricuspid regurgitation with competent aortic and mitral valves. She progressively gained weight in hospital despite diuretics and infusion of dopamine. We decided to perform further tricuspid valve surgery, and to improve her clinical state haemofiltration was performed for 48 hours before surgery.

She underwent venovenous haemofiltration with a BSM-22 (Hospal UK), with vascular access through a right internal jugular haemofiltration catheter; a Biospal SCU/CAVH filter was used. About 16 litres of haemofiltrate was obtained on each day, with a negative balance of four litres. Fluid replacement through the BSM-22 was with Hemofiltrasol-21, with 8 mmol potassium chloride added to each three litre bag. In addition, she also received two units of human plasma protein fraction for the first 24 hours, 20% mannitol 100 ml intravenously twice and frusemide 80 mg intravenously once in the second 24 hours, and spironolactone 100 mg once daily.

Her clinical state improved. To our surprise her plasma sodium concentration decreased progressively from 138 mmol/l at the start of the procedure to 133 mmol/l after 48 hours of haemofiltration. Forty eight hours after haemofiltration had stopped her plasma sodium concentration remained at 134 mmol/l. Blood glucose concentration rose from 5·2 mmol/l (random level) before treatment to 8·4 mmol/l during haemofiltration. The plasma potassium concentration, supplemented as described above, remained at 3·8 mmol/l. The serum calcium concentration rose from 2·37 mmol/l to 2·60 mmol/l. There was no appreciable change in the magnesium concentration.

We were surprised by the progressive decrease in plasma sodium concentration during haemofiltration. The Hemofiltrasol-21 bag quoted the sodium concentration as being 140 mmol/l. When a sample of this was analysed on the same flame photometer as the plasma it was found to contain 133 mmol sodium/l. This was confirmed in two other bags of the same batch.

The sodium concentration of 133 mmol/l is within the limits of quality control of Hemofiltrasol-21. We therefore investigated the limits of quality control fluids from four suppliers in this country. The limits varied widely between manufacturers—for example, the limits of quality control for sodium with one manufacturer were +3% to −3%, while with another they were +5% to −7%. Those of magnesium were +5% to −5% with one manufacturer and +13% to −13% with another. The table shows the stated concentration and the limits of some ions.

Concentration (mmol/l) of some common ions in haemofiltration fluids (figures in parentheses are limits of quality control)

Supplier	Sodium	Calcium	Magnesium	Chloride
1	142 (138-146)	2·00 (1·90-2·10)	0·75 (0·71-0·79)	103·0 (98·0-108·0)
2	140 (130-147)	1·60 (1·40-1·80)	0·75 (0·68-0·83)	100·0 (90·0-110·0)
3	135 (128-142)	1·88 (1·79-1·97)	0·75 (0·71-0·79)	106·5 (101·2-111·8)
4	135 (128-142)	1·20 (1·10-1·30)	0·75 (0·65-0·85)	103·0 (98·0-108·0)

Comment

The development of hyponatraemia in heart failure is associated with poor prognosis.[1] The wide limits of quality control applied to some ion concentrations make control of electrolyte balance during haemofiltration unpredictable and potentially hazardous, especially when large volumes are infused. Fluids from suppliers 3 and 4 are within 2% of their intended ion concentrations at the time of manufacture, though they apply wider limits of quality control at the end of the shelf life owing to the evaporation of water from polyvinylchloride bags.

Doctors must remain alert to electrolyte imbalance caused by variations in fluid constituents during large intravenous infusions. Medicinal products without a product licence are prepared as "specials," made to the clinician's specification. Until a manufacturer obtains a product licence doctors who require haemofiltration fluids should specify not only the required concentration of ions but also the acceptable limits of quality control.

1 Lee WH, Packer M. Prognostic importance of serum sodium concentration and its modifications by converting enzyme inhibition in patients with severe chronic heart failure. *Circulation* 1986;73:257-67.

(Accepted 17 August 1987)

National Heart Hospital, London W1M 8BA
N D BARBER, PHD, MPS, staff pharmacist in charge
J S R GIBBS, MA, MRCP, honorary registrar
P BARRETT, BSC, MPS, pharmacist
K M FOX, MD, MRCP, consultant cardiologist

Correspondence and requests for reprints to: Dr Gibbs.

Increased cough reflex associated with angiotensin converting enzyme inhibitor cough

Angiotensin converting enzyme inhibitors are being used increasingly to treat hypertension and congestive cardiac failure as they are one of the few treatments that improve life expectancy in these conditions.[1] In general, these drugs are well tolerated, but recently unexpected and troublesome cough, without obvious pulmonary abnormality, has been reported in as many as 5-10% of patients taking both captopril and enalapril.[2] Until recently cough has been difficult to assess, but we have developed a method for measuring the sensitivity of the cough reflex to a standard inhaled stimulus: capsaicin (red pepper).[3] Using a modification of this method, we assessed the sensitivity of the cough reflex in five patients with cough associated with treatment with angiotensin converting enzyme inhibitors.

Epidem. Inf. (1987) 99, 579–589
Printed in Great Britain

An outbreak of meningococcal disease in Stonehouse: planning and execution of a large-scale survey

By J. M. STUART.

Department of Community Medicine, Rikenel, Gloucester GL1 1LY

K. A. V. CARTWRIGHT.

Public Health Laboratory, Gloucester Royal Hospital, Great Western Road, Gloucester GL1 3NN

D. M. JONES.

Public Health Laboratory, Withington Hospital, Manchester M20 8LR

N. D. NOAH.

PHLS Communicable Disease Surveillance Centre, Colindale, London NW9 5EQ

R. J. WALL.

Clinical Research Centre, Northwick Park, Harrow, London

C. CAROLINE BLACKWELL.

Department of Bacteriology, University of Edinburgh, Teviot Place, Edinburgh

A. E. JEPHCOTT

Public Health Laboratory, Myrtle Road, Kingsdown, Bristol BS2 8EL

AND I. R. FERGUSON

Public Health Laboratory, County Hospital, Hereford HR1 2ER

(*Accepted 18 August 1987*)

SUMMARY

In November 1986 a large-scale survey was undertaken in the Gloucestershire town of Stonehouse during an outbreak of meningococcal disease due to group B type 15 subtype P1.16 sulphonamide-resistant strains. There were 15 cases in Stonehouse residents during the 4 years from April 1983, an annual attack rate of 56·5 per 100000. Four secondary cases occurred despite rifampicin prophylaxis. The objectives of this community survey were to investigate patterns of meningococcal carriage, transmission and immunity and to determine the proportion of non-secretors of blood group antigens in the Stonehouse population and amongst meningococcal carriers. A total of 6237 subjects participated including 75% of the 6635 Stonehouse residents. Over 97% of the participants provided all three of the requested specimens – nasopharyngeal swabs, saliva and blood samples.

The co-operation between the many organizations involved in the detailed

preliminary planning was instrumental in the success of the survey; in particular the value of effective collaboration between Departments of Community Medicine and Microbiology and of the Public Health Laboratory Service network of laboratories in undertaking investigations of this size and type was clearly demonstrated.

INTRODUCTION

Since 1974 meningococcal disease due to group B type 15 p1.16 sulphonamide-resistant (B15.16R) strains has been encountered increasingly in several European countries including Britain (Poolman *et al.* 1986). It has been characterized by prolonged outbreaks with high attack rates in teenagers and young adults and a low carriage rate in community contacts (Cartwright, Stuart & Noah, 1986). These unusual features of meningococcal disease suggest that B15.16R strains have a greater virulence, longer duration of carriage and lower transmissibility than most other strains of meningococci and that they may not have circulated in the affected communities for 20 years or more.

The factors influencing susceptibility of the non-immune individual to meningococcal disease are not fully understood though recent studies have shown that a genetically determined inability to secrete ABO blood group antigens in saliva and other body fluids (non-secretion) is one such predisposing factor (Blackwell *et al.* 1986).

In south-west England both Gloucester and Plymouth Health Districts have experienced prolonged outbreaks of meningococcal disease due to B15.16R strains since 1981 (Cartwright, Stuart & Noah, 1986; Dawson, Rickard & Wilkinson, 1986). In Gloucester Health District (population 308·500) 94 cases occurred between 1 October 1981 and 31 March 1987, an average annual attack rate of 5·5 per 100 000. Of these cases 15 occurred in the parish of Stonehouse (population 6635), a town in Stroud Local Authority District. Eleven of the 15 cases lived on one estate, which housed 26% of the town's population.

This report describes the outbreak in Stonehouse and the planning and execution of a large scale study which had the following principal objectives:

1. To measure the prevalence of nasopharyngeal carriage of, and immunity to, B15.16R and other strains of meningococci in the Stonehouse population by age, sex, school and area of residence during an outbreak of meningococcal disease caused by B15.16R strains.

2. To examine the hypothesis that B15.16R meningococci have a relatively low transmissibility and long duration of carriage.

3. To determine the proportion of non-secretors in the Stonehouse population and amongst meningococcal carriers.

The bacteriological findings are described in the companion article (Cartwright *et al.* 1987). The serological and secretor results will be described in further papers.

METHODS

Planning

Planning meetings were held during the summer and autumn of 1986. Six of the authors (J.S., K.C., D.J., N.N., R.W., C.B.) constituted the core planning team.

The local planning team was made up of one nursing, one administrative and one finance officer, a general practitioner, a computer programmer, two head medical laboratory scientific officers (MLSOs), a county education officer, a community physician and three Public Health Laboratory (PHL) directors. The laboratory planning team consisted of the Directors and Head MLSOs of Gloucester, Manchester, Bristol and Hereford PHLs.

Timing

The duration of the survey needed to be as short as practicable so that an estimate of point prevalence of meningococcal carriage and immunity could be obtained. It was also considered important to complete the survey before the end of 1986 in order to study the community during a period of high disease activity. The two weeks beginning 3 November 1986 were chosen: sampling sessions were planned on weekday evenings and Saturdays at Stonehouse Health Clinic and at Stonehouse schools during the week days.

Sample size

A low carriage rate of B15.16R organisms (1% or less) was anticipated. The uneven distribution of the cases made it important to compare the prevalence of immunity, carriage rates and secretor state in different areas of the town. With the exception of infants who were excluded for practical reasons, all residents of Stonehouse Parish were invited to participate. All children and staff at Stonehouse schools, whether or not Stonehouse residents, were included so that comparisons could be made between schools. The total sample size was estimated as 7700 from Gloucestershire Family Practitioner Committee (FPC) records and school lists.

Specimens

Postnasal swabs were needed to determine rates of nasopharyngeal carriage of meningococci, saliva samples to determine secretor status, and blood samples to validate the secretor results and to detect specific IgM and IgG antibodies against B15.16R and other meningococci by ELISA tests.

Staff and equipment

A 'dummy run' was held at the Health Clinic. Estimates were made of the time to complete sampling, the staff and equipment needed, and costs. Calculations were based on a 90% attendance rate with a maximum throughput of 90 people per hour in each evening session and 60 per hour in each school session. Medical, nursing, laboratory, administrative and clerical staff were recruited and equipment was ordered pending approval of funding.

Ethical issues

Approval for the study was obtained from the District Ethical Committee and medico-legal advice was sought on wording of consent forms.

Publicity

Plans of the survey giving details of the reasons for its undertaking, the dates, and further general information were made widely available through the local media.

Representatives of the national press and television were invited to a press conference on the first morning of the survey. This increased local awareness and allowed the survey to proceed thereafter without disruption from the media. The Press Office of the South Western Region Health Authority handled enquiries during the survey.

Implementation

The Meningitis Trust, a locally based charity, arranged for the separate delivery of two explanatory letters, the second with four registration cards, to each household in Stonehouse Parish. The registration cards included details of surname, forename, date of birth, sex, address, postcode, general practitioner, school and class (where relevant) and signed consent.

The 1985 electoral register was used to divide the town into 10 localities with approximately equal adult populations. Householders were asked if possible to attend Stonehouse Health Clinic with their completed cards on one of the 10 designated weekday evenings between 4 p.m. and 9 p.m. Children of pre-school age or at school outside Stonehouse were also invited to these evening sessions. Residents who were unable to attend during a weekday evening were asked to come between 9 a.m. and 6 p.m. on one of the two Saturdays.

The head teachers of the Stonehouse schools issued a letter to parents and a registration card to each pupil prior to the start of the survey. Pupils were asked to take the letter and card home, complete the card, obtain signed parental consent (if aged less than 16) and return the completed card to form teachers. Days and times for sampling at schools were agreed with head teachers.

Three teams, each consisting of one clerical assistant, three nurses, one MLSO and one phlebotomist, collected the specimens at each evening session: there were two teams at the schools. In addition there was an administrator assisted by volunteers to control flow of subjects to the teams, a medical officer to deal with emergencies and to assist in taking blood samples, the two co-ordinators (K.C. and J.S.) to supervise and to assist in specimen collection, a volunteer from the Red Cross to administer first aid, a clerical officer to enter registration data on to a computer and a driver to transport specimens. Each team had the use of three rooms – the first for checking data registration and for saliva collection, the second for throat swabbing and plating and the third for blood sampling.

Room One: A clerical officer helped to complete registration cards where necessary and checked that data entry was complete and legible. Postcodes were cross-checked against addresses on a Post Office list. Six adhesive labels bearing an identical number were used to identify the registration card and all the specimens from one subject. No other specimen identification system was used.

Saliva specimens (2 ml) were collected by a nurse. Subjects expectorated into 20 ml sterile Universal containers; plastic disposable pipettes were used when necessary in young children. A flow of saliva was stimulated by smelling fresh cut lemons. Samples were frozen to −20 °C within 3 h of collection and subsequently transferred to Edinburgh in insulated containers.

Room Two: A nurse swept the posterior pharyngeal wall behind the uvula with a fluffy charcoal swab. Pernasal swabs were used if necessary in small children. Plates were inoculated directly by an MLSO and taken by van in alternate 3 h batches to Gloucester and Bristol PHLs.

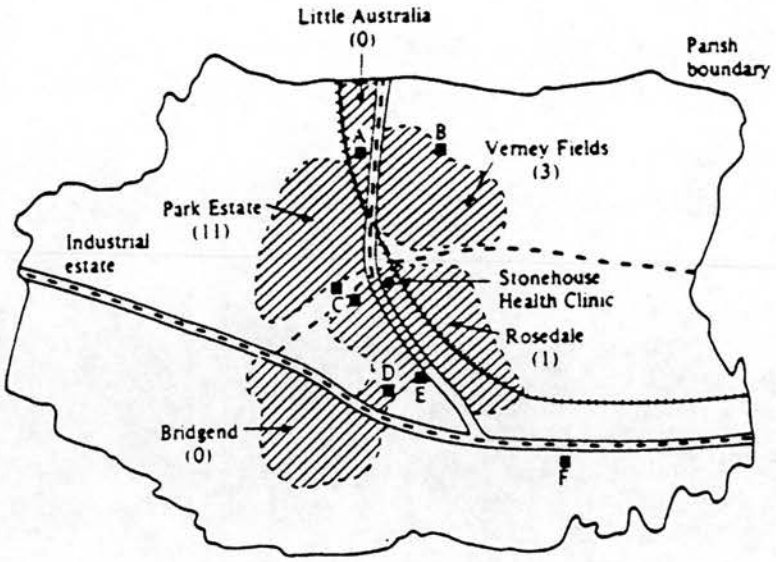


Fig. 1. Map of Stonehouse. Key to schools: A. Special: B. state secondary: C. state infant and junior: D. private junior (*a*): E. private senior: F. private junior (*b*). Numbers of cases of meningococcal disease are shown in parentheses. ----- Boundaries between areas.

Table 1. *Meningococcal disease in Stonehouse residents: case details*

Case number	Disease onset (month/year)	Age at onset	Sex	Area of residence	School	Organism isolated
1	5/83	46	M	PE	—	B15:P1.16R
2	3/84	10	M	PE	State junior	B15:P1.16R
3	9/84	12	F	PE	State secondary	B2bR
4	12/85	11	F	PE	Outside Stonehouse	CSF film positive
5	12/85*	6	M	PE	State infant	B15:P1.16R
6	1/86*	11	F	PE	Special	B15R
7	3/86**	5	M	PE	State infant	B15:P1.16R
8	6/86**	9	F	PE	State junior	B15:P1.16R
9	7/86*	32	M	PE	—	B15:P1.16R
10	7/86***	4	M	VF	—	Clinical diagnosis
11	7/86 (Died)	7	M	PE	State infant	Clinical diagnosis
12	7/86	6	M	VF	State infant	Clinical diagnosis
	Survey November 1986					
13	12/86 (Died)	7	M	PE	State junior	B15:P1.16R
14	12/86***	2	M	VF	—	B15:P1.16R
15	3/87	9	F	R	State junior	B15:P1.16R

*, **, *** indicate cases in same households.
PE. Park Estate: VF. Verney Fields: R. Rosedale.

estimated 20·7% in Stroud District: the proportions in Park Estate and Verney Fields were close to the mean for Stonehouse (Table 2). The proportion of people aged 65 and over was 17·6% in Stonehouse compared with an estimated 16·0% in Stroud District; of the five Stonehouse areas Park Estate had the highest figure at 20·6%.

Table 2. *Population and age distribution in Stonehouse*

Population	Park Estate	Verney Fields	Little Australia	Rosedale	Bridgend	Total
Total	1729	1836	869	1423	778	6635
< 16 years (%)	26·3	27·9	31·8	26·8	24·9	27·4
> 64 years (%)	20·6	20·1	8·8	15·9	18·1	17·6

Sources: Glos. F.P.C.: Stroud District Council.

Table 3. *Housing, population density and occupation in Stonehouse*

	Park Estate	Verney Fields	Little Australia	Rosedale	Bridgend	Total
No. of houses	641	760	370	541	289	2610
Council-owned (%)	56·5	57·2	0	5·2	17·5	33·6
Mean no. people per house	2·7	2·4	2·4	2·6	2·6	2·5
Mean no. people per acre	26·6	24·6	28·8	14·6	12·1	20·0
Household heads with manual occupation (%)	77·5	74·1	51·7	46·5	65·0	63·7

Sources: Glos. F.P.C.: Stroud District Council: 1981 Census.

Boundaries of enumeration districts did not coincide with the boundaries of the designated areas. An analysis of the 1981 Office of Population Census and Surveys Small Area Statistics showed that in those enumeration districts most closely corresponding to Park Estate and Verney Fields there was a higher incidence of employed household heads in Social Classes IIIM, IV and V compared with the rest of Stonehouse (Table 3). Another socio-economic indicator, the proportion of council-owned housing, was 33·6% in Stonehouse at the time of the survey compared with 17·8% in Stroud District. Park Estate and Verney Fields contained 91% of council-owned housing in Stonehouse. There was little variation in the average number of people per house though Park Estate had the highest figure; estimates of population density within the residential parts of each area averaged 20 people per acre, the highest density being in Little Australia (Table 3).

During the fortnight of the survey 6237 persons attended for screening: 5006 were Stonehouse residents and the remaining 1231 were non-resident pupils and staff of Stonehouse schools. This represented a 75% attendance by the residents of Stonehouse and included 87% of the 1–19-year-olds (Table 4). The under 1-year-olds had not been invited but 16 (15%) were tested on request by their parents.

The lowest attendance from the five areas was 66% from Verney Fields (Table 5). All the schools had very high attendance figures including those pupils not living in Stonehouse. The overall percentage of school-children tested was 96% (Table 6). Technical failures in testing or refused consent were uncommon – 99·8% of the 6237 attenders provided a nasopharyngeal swab, a saliva sample was collected from 98·9% and a blood sample from 97·5% (Table 7). Even in the 1–4-year age group venepuncture was successful in 87%.

Table 4. *Attendance of Stonehouse residents by age and sex*

Age in years	Males			Females		
	Population	Tested		Population	Tested	
		No.	(%)		No.	(%)
< 1	59	6	(10·2)	46	10	(21·7)
1–4	214	179	(83·6)	169	133	(78·7)
5–9	237	226	(95·4)	198	195	(98·5)
10–14	182	181	(99·5)	193	186	(96·4)
15–19	261	187	(71·6)	256	201	(78·5)
20–24	257	177	(68·9)	257	209	(81·3)
25–34	492	388	(78·9)	533	424	(79·5)
35–44	421	324	(77·0)	418	354	(84·7)
45–54	298	216	(72·5)	295	226	(76·6)
55–64	300	214	(71·3)	381	296	(77·7)
65+	475	268	(56·4)	693	406	(58·6)
Total	3196	2366	(74·0)	3439	2640	(76·8)

Table 5. *Attendance of Stonehouse residents by area*

	Population	Tested	
		No.	(%)
Park Estate	1729	1273	(73·6)
Verney Fields	1836	1216	(66·2)
Little Australia	869	750	(86·3)
Rosedale	1423	1226	(86·2)
Bridgend	778	541	(69·5)
Total	6635	5006	(75·4)

Table 6. *Attendance rate of children at school in Stonehouse*

School*	No. of children on school roll			Pupils tested	
	Total	Resident in Stonehouse			
		No.	(%)	No.	(%)
State infant/junior	443	428	(96·6)	441	(99·1)
Private junior (a)	49	4	(8·2)	49	(100·0)
Private junior (b)	194	19	(9·8)	180	(92·8)
State secondary	781	280	(35·9)	736	(94·2)
Private senior	337	14	(4·2)	331	(98·2)
Special	58	5	(8·6)	51	(87·9)
Total	1862	750	(40·3)	1788	(96·0)

* See Fig. 1.

Table 7. *Sampling compliance in all attenders*

Age group in years	Total no. of attenders	Postnasal swab samples		Saliva samples		Blood samples	
		No.	(%)	No.	(%)	No.	(%)
1–4	324	324	(100·0)	296	(91·4)	282	(87·0)
5–9	537	535	(99·3)	531	(98·9)	515	(95·9)
10 & over	5376	5375	(100·0)	5340	(99·3)	5285	(98·3)
Total	6237	6234	(99·9)	6167	(98·9)	6082	(97·5)

Throughput sometimes reached the planned maximum of 90 subjects per hour but waiting rarely exceeded 15 min at any time. The school testing also kept to schedule.

One brief grand mal seizure was recorded in a boy with no history of fits: there were no other serious adverse events.

DISCUSSION

Interesting features of the Stonehouse outbreak include the localization of the disease within the town, the high attack rate in 5- to 9-year-old children and the high secondary attack rates.

It is difficult to obtain an accurate age–sex structure of any defined population between each 10-year census. Electoral registers are updated annually but provide only a list of residents eligible to vote. Consequently it was considered that the computerized FPC registration list, despite inaccuracies due to logistic delays, would provide the most accurate estimate of the Stonehouse population without conducting a separate household census. Although Stonehouse had a 7% higher proportion of under-16-year-olds than the mean for the Stroud District, the proportions of under-16-year-olds (and of 5- to 9-year-olds) in Park Estate and Verney Fields were close to the mean for Stonehouse so that the higher disease rates in these areas could not be explained by a predominance of the more susceptible age groups. The mean number of people per house was marginally higher in Park Estate but the population density was higher in Little Australia. The main associations found in this analysis were that the two areas in which all but one of the cases lived had considerably higher proportions of council-owned housing and of correspondingly lower social class in household heads than the rest of the town. Previous research has also shown an association between lower socio-economic indicators and meningococcal disease (Farries *et al.* 1975).

Rifampicin prophylaxis for household contacts of a case of meningococcal disease is recommended in Britain and North America in order to reduce secondary attack rates (PHLS, 1986; Centers for Disease Control, 1981). Compliance is likely to be good in the family members of a child with meningococcal disease. The high secondary attack rate reported here is disappointing and raises doubts about the effectiveness of rifampicin in preventing secondary cases. The long delay between index and secondary cases could be explained by the time taken for the virulent strain to be reintroduced into the family and thence to reach another susceptible family member; rifampicin may do

Table 4. *Attendance of Stonehouse residents by age and sex*

Age in years	Males			Females		
	Population	Tested		Population	Tested	
		No.	(%)		No.	(%)
< 1	59	6	(10·2)	46	10	(21·7)
1–4	214	179	(83·6)	169	133	(78·7)
5–9	237	226	(95·4)	198	195	(98·5)
10–14	182	181	(99·5)	193	186	(96·4)
15–19	261	187	(71·6)	256	201	(78·5)
20–24	257	177	(68·9)	257	209	(81·3)
25–34	492	388	(78·9)	533	424	(79·5)
35–44	421	324	(77·0)	418	354	(84·7)
45–54	298	216	(72·5)	295	226	(76·6)
55–64	300	214	(71·3)	381	296	(77·7)
65 +	475	268	(56·4)	693	406	(58·6)
Total	3196	2366	(74·0)	3439	2640	(76·8)

Table 5. *Attendance of Stonehouse residents by area*

	Population	Tested	
		No.	(%)
Park Estate	1729	1273	(73·6)
Verney Fields	1836	1216	(66·2)
Little Australia	869	750	(86·3)
Rosedale	1423	1226	(86·2)
Bridgend	778	541	(69·5)
Total	6635	5006	(75·4)

Table 6. *Attendance rate of children at school in Stonehouse*

School*	No. of children on school roll			Pupils tested	
	Total	Resident in Stonehouse			
		No.	(%)	No.	(%)
State infant/junior	443	428	(96·6)	441	(99·1)
Private junior (a)	49	4	(8·2)	49	(100·0)
Private junior (b)	194	19	(9·8)	180	(92·8)
State secondary	781	280	(35·9)	736	(94·2)
Private senior	337	14	(4·2)	331	(98·2)
Special	58	5	(8·6)	51	(87·9)
Total	1862	750	(40·3)	1788	(96·0)

* See Fig. 1.

no more than defer the onset of disease in close contacts of cases. The long interval between primary and secondary cases adds further weight to the hypothesis that B15.16R organisms are less transmissible than other strains of pathogenic meningococci.

Although there have been extensive studies of meningococci in military populations, particularly in the USA (Goldschneider, Gotschlich & Artenstein, 1969), no study on this scale has been previously undertaken to investigate meningococcal carriage and immunity in a civilian population. The relationship between non-secretion and meningococcal carriage has not been examined previously.

There was a very high overall attendance and nearly all who came to a sampling session provided all three requested samples. Several factors may have contributed to the high attendance. First, meningococcal disease can be frightening in its severity. It often begins suddenly in previously healthy individuals and may cause death within a few hours. A case in a school causes intense anxiety in pupils, parents and teachers, whereas cases occurring in infants generally receive less publicity. As several cases had occurred recently in school-children living in Stonehouse, parents and school-children were all well aware of the problem. Second, enlisting the support of the local and national media ensured that interest was maintained at a high level. Third, individual explanatory letters to each household accompanied by registration cards ensured that all individuals were aware that their active participation was being sought. Fourth, the provision of an adequate number of sampling sessions ensured that processing of subjects was rapid and that delays were minimal. The co-operation of the school authorities in this respect was invaluable in providing facilities for sampling at the individual schools and in releasing children class by class to attend.

The high compliance in providing all three specimens was primarily a reflection of the willingness of nearly all the subjects to help. An additional factor was the use of a single signed consent for all three tests. Separate rooms for the sampling procedures assisted in reducing anxiety and in improving compliance in blood testing. Only a few individuals declined to provide the full set of specimens. The main reason for not achieving even higher compliance for blood collection was occasional technical failure, particularly in very young children. Nevertheless it is possible, with the co-operation of parents, to collect blood samples from the majority of children as young as 1 year of age.

The survey was completed within the planned 2-week period, and continuing disease activity was confirmed by the occurrence of the three further cases.

Early recruitment of nurses, phlebotomists, technical, clerical and medical staff and their subsequent briefing meant that all the teams were well informed about the methods and objectives of the survey. Sharing of data in the collaborating centres was made possible by the use of compatible computer hardware and by the early involvement of PHLS Computer Services in the design of programs. The division of the laboratory work between four Public Health Laboratories proved essential in managing the large workload additional to the routine services. The high attendance and high compliance for all three sampling procedures emphasized the importance of detailed preliminary planning. Effective collaboration between the Departments of Community Medicine and Microbiology and within the network of PHLS laboratories was vital to the success of this survey.

We gratefully acknowledge the willing assistance of the population of Stonehouse, the headmasters, staff and pupils of Stonehouse schools, voluntary helpers and all the professional, technical and clerical staff involved in the collection and handling of specimens and data. We also thank Professor J. R. T. Colley for advice on the organization of the survey.

The survey was supported by the D.H.S.S. and by the Meningitis Trust.

REFERENCES

BLACKWELL, C. C., JONSDOTTIR, K., HANSON, M., TODD, W. T. A., CHAUDHURI, A. K. R., MATHEW, B., BRETTLE, R. P. & WEIR, D. M. (1986). Non-secretion of ABO antigens predisposing to infection by *Neisseria meningitidis* and *Streptococcus pneumoniae*. *Lancet* ii. 284–285.

CARTWRIGHT, K. A. V., STUART, J. M., JONES, D. M. & NOAH, N. D. (1987). The Stonehouse survey: nasopharyngeal carriage of meningococci and *Neisseria lactamica*. *Epidemiology and Infection* 99. 591–601.

CARTWRIGHT, K. A. V., STUART, J. M. & NOAH, N. D. (1986). An outbreak of meningococcal disease in Gloucestershire. *Lancet* ii. 558–561.

CENTERS FOR DISEASE CONTROL (1981). Meningococcal disease – United States. *Morbidity and Mortality Weekly Report* 30. 113–115.

DAWSON, J. A., RICKARD, J. & WILKINSON, P. J. (1986). Meningococcal disease in south-west of England. *Lancet* ii. 806–807.

FARRIES, J. S., DICKSON, W., GREENWOOD, E., MALHOTRA, T. R., ABBOTT, J. D. & JONES, D. M. (1975). Meningococcal infections in Bolton, 1971–74. *Lancet* ii. 118–121.

GOLDSCHNEIDER, I., GOTSCHLICH, E. C. & ARTENSTEIN, M. S. (1969). Human immunity to the meningococcus. I. The role of humoral antibodies. *Journal of Experimental Medicine* 129. 1307–1326.

PHLS COMMUNICABLE DISEASE SURVEILLANCE CENTRE (1986). Report. *British Medical Journal* 293. 1293–1294.

POOLMAN, J. T., LIND, I., JONSDOTTIR, K., FROHOLM, L. O., JONES, D. M. & ZANEN, H. C. (1986). Meningococcal serogroups and serogroup B disease in north-west Europe. *Lancet* ii. 555–557.

Non-secretion of blood group antigens. A genetic factor predisposing to infection by *Neisseria meningitidis*

C. C. BLACKWELL, K. JONSDOTTIR[1], I. MOHAMMED[2] & D. M. WEIR

Dept. of Bacteriology, University of Edinburgh, Scotland, UK; [1] Dept. of Bacteriology, University of Iceland, Iceland; [2] Dept. of Medicine, University of Maiduguri, Nigeria

Abstract. We have identified a genetic factor associated with susceptibility to bacterial meningitis. This is the inability of an individual to secrete the water-soluble glycoprotein form of the ABO blood group antigen. Compared with local controls, a significant increase in the proportion of non-secretors was observed among patients with infection due to *Neissereria meningitidis* in Scotland, Iceland and northern Nigeria.

Investigation of the host-parasite interactions responsible for these epidemiological observations might provide valuable insights into the pathogenesis of these infections.

Introduction

Efforts to produce vaccines against *Neisseria meningitidis* have focused on virulence factors of the microorganism. Our knowledge of stable host factors that predispose the non-immune individual to these infections is much more limited. Distribution of the ABO blood groups and alterations in the predicted frequencies of secretors and non-secretors of blood group antigens in various populations suggested that these factors might influence susceptibility or resistance to infectious agents (Mourant 1982, 1983). There is an increased incidence of non-secretors among patients with cholera (Chaudhuri & DasAdhikary 1978), recurrent urinary tract infections (Blackwell et al. 1986b) superficial candida infections (Blackwell et al. 1986c), invasive pneumococcal disease and meningococcal infection (Blackwell at al. 1986a).

The ability of an individual to secrete the water-soluble glycoprotein form of his ABO blood group antigens into body fluids is a stable character inherited in a Mendelian-dominant pattern. The recessive non-secretor phenotype is predicted for about 25% of any population. Because this figure can vary in different ethnic groups (Thordarson et al. 1972; Chown & Lewis 1955), local controls

J. T. Poolman et al. (Eds) Gonococci and Meningococci. ISBN 90-247-3607-2

are very important. Secretor state does not alter with age or environmental influences, so retrospective studies can be carried out.

Materials and methods

Secretor state was determined by the method described by Mollison (1979). Local controls were blood donors from the Edinburgh area, blood donors and hospital personnel in Iceland, women attending antenatal clinics at Maiduguri, Nigeria. Specimens were obtained from household contacts of the Icelandic patients. The blood groups of the Nigerian populations were also supplied.

Results

In each of the 3 different populations studied there was a significant increase in the incidence of non-secretors among the individuals with meningococcal infections compared with local controls (Table 1).

Because the meningitis belt of Africa can be superimposed on the area in which there is the highest incidence of the B gene (Winstanley et al. 1985), we have predicted there might also be an increase in the incidence of B and/or AB individuals with meningitis. There was no significant differences between patient and control groups (Table 2).

Among the 26 contacts of Icelandic patients tested, 11 were secretors and 15 non-secretors. Because older children might have developed protective antibodies, we analyzed the contacts by age and secretor state. When the ages of the contacts younger that the patient were compared, the mean age of the secre-

Table 1. Incidence of non-secretors among patients with meningococcal infection.

Source	Total	Non-secretor		p
		No.	(%)	
Scotland				
Controls	334	89	(26.6)	< .005
Patients	26	18	(69)	
Iceland				
Controls	228	94	(41.2)	< 0.05
Patients	98	53	(54)	
Nigeria				
Controls	186	92	(49.5)	< 0.01
Patients	42	31	(73.8)	

Table 2. Distribution of ABO blood groups for Nigerian controls and patients.

	ABO blood group			
Source	A (%)	B (%)	O (%)	AB (%)
Antenatal clinic	34 (18)	42 (23)	103 (55)	7 (4)
Meningitis patients	10 (24)	9 (21)	20 (48)	3 (7)

Table 3. Secretor state of uninfected contacts of Icelandic patients with meningococcal disease.

	Secretors	Non-secretors
Older than patient	4	7
Younger than patient	7	8
*Mean age of younger patients	1.3	3.2

*$0.1 > p > 0.05$

tors was 1.3 years and that of the non-secretors 3.2 years ($0.1 > p < 0.05$). The numbers are small, but the t value was just outside the 95% confidence limits (Table 3).

Discussion

In comparison with local controls, there was a significant increase in the incidence of non-secretors among patients with meningococcal infections, even in Iceland and the area around Maiduguri where the incidence of this recessive character is unusually high. These results suggest that the ability to secrete blood group antigens is part of the host's innate defences, and this protective effect is observed particularly in non-immune (Blackwell et al. 1986a) or immunocompromised persons (Blackwell et al. 1986c). Among the contacts of Icelandic patients, differences in the ages of the secretors and non-secretors suggest that secretion might help protect very young children during the critical period between decline of maternal antibodies and development of child's own active immunity to these pathogens.

We have suggested 2 hypothesis to explain the host-parasite interactions underlying these epidemiological observations:

1. There are glycocompounds in the body fluids of secretors that can inhibit binding of lectin-like adhesins on the surface of microorganisms thus reducing the probability of colonization.

2. The Lewis^a antigen found on the cells of non-secretors is one of the receptors for adhesins on the microorganisms.

There is evidence for the first hypothesis from our work on candida (Blackwell et al. 1986c). We were able to demonstrate inhibition of binding by preincubation of the yeast with boiled saliva from secretors but not non-secretors.

Evidence for the second hypothesis comes again from our studies of candida. Binding of the yeast was inhibited if non-secretor epithelial cells were pretreated with anti-Lewis^a antisera, but no inhibition was observed following treatment of secretor cells with anti-Lewis^b antisera (May et al. 1986).

By identifying host receptors we plan to exploit this information to isolate adhesins from these microorganisms and to examine their immunogenicity to evaluate their potential as vaccine candidates.

Acknowledgements

We are grateful to the Edinburgh and South East Scotland Blood Transfusion Service for reagents used in this study and to the Meningitis Trust for travel support.

References

Blackwell, C. C., K. Jonsdottir, M. Hanson, W. T. A. Todd, A. K. R. Chaudhuri, B. Mathew, R. P. Brettle & D. M. Weir (1986a) Non-secretion of ABO antigens predisposing to infection by *Neisseria meningitidis* and *Streptococcus pneumoniae*. Lancet ii: 284–285

Blackwell, C. C., S. J. May, R. P. Brettle, C. J. MacCallum & D. M. Weir (1986b) Host-parasite interactions underlying non-secretion of blood group antigens and susceptibility to recurrent urinary tract infections. In: D. Lark (Ed) Protein-Carbohydrate Interactions in Biological Systems (pp. 229–230). Academic Press, London

Blackwell, C. C., S. M. Thom, O. Lawrie, D. M. Weir, D. Wray & D. F. Kinane (1986c) Non-secretion of blood group antigens and susceptibility to oral infection due to *Candida albicans*. J. Dent. Res. 65: 502

Chaudri A. & C. R. DasAdkikary (1978) Possible role of blood group secretory substances in the aetiology of cholera. Trans. Roy. Soc. Trop. Med. Hyg. 72: 664–665

Chown, B. & M. Lewis (1955a) The blood groups and the secretor genes of the Stoney and Sarcee Indians of Alberta, Canada. Amer. J. Phys. Anthrop. N.S., 13: 181–189

May, S. J., C. C. Blackwell & D. M. Weir (1986) Non-secretion of blood group antigens and susceptibility to *Candida albicans*: the role of Lewis blood group antigens. J. Den. Res. 65: 503

Mourant, A. E. (1983) Blood Relations. Blood Groups and Anthropology Oxford University Press, Oxford

Thordarson, G., C. Bjarnason, P. J. Lincoln & B. E. Dodd (1972) ABH secretor and Lewis type frequencies in an Icelandic series. J. med. Genet. 9: 46–47

Winstanley, F. P., C. C. Blackwell & D. M. Weir (1985) Factors influencing susceptibility to meningococcal diseases. Biomedicine and Pharmacotherapie 39: 167–170

pedal cyclists, 98 (19%) had mild concussion, 13 (3%) had severe head injuries, and 58 (11%) had cuts and grazes.

The Oxford ring road encloses 227 km of road and only 23 km of cycle lane, of which 8 km are part of the road and 15 km are separate. The number of injuries per kilometre was 0·9 on the road, 0·4 on the road cycle lane, and 0·3 on the separate cycle track. Another vehicle caused or was involved in 1012 (55%) of the accidents.

Comment

In accidents motorcyclists have been shown to sustain more severe injuries to the body and pedal cyclists more severe head injuries,[1] a finding confirmed by our study. Although the two groups are not strictly comparable, we suggest that if cyclists wore helmets the number of head injuries in this group would be reduced. The incidence of head injuries sustained by pedal cyclists is similar whatever their age.[2] Though children form the largest group of cyclists with head injuries, it would be desirable for all cyclists to wear helmets.

Our study suggests that cycle lanes are safer than ordinary roads for cyclists. After the introduction of cycle lanes on certain routes in Oxford the number of accidents remained the same despite double the number of road users (unpublished report of city engineer). It has been suggested that cycle lanes relocate bicycle accidents to the place where the lanes end[3]; thus special cycle routes using quieter parts of the existing road system should perhaps be developed. All the deaths in our study and 87% of those in the study by Nixon *et al*[1] occurred after an accident with a motor vehicle and could have been averted by complete segregation of cyclists. Although this aim is currently impracticable because of the dominance of cars, cycle lanes and especially separate cycle tracks are a worthwhile addition to city centres.

1 Nixon J, Clacher R, Pearn J, Corcoran A. Bicycle accidents in childhood. *Br Med J* 1987;294: 1267-9.
2 McDermott FT, Klug GL. Head injury predominance: pedal-cyclists vs motor-cyclists. *Med J Aust* 1985;143:232-4.
3 Anonymous. Motorcycle and bicycle accidents. *Br Med J* 1979;i:39-41.

(Accepted 12 February 1987)

Nuffield Department of Orthopaedic Surgery, Accident Service, John Radcliffe Hospital, Oxford OX3 9DU
A H R W SIMPSON, MA, FRCS, orthopaedic registrar
P S UNWIN, BSC, research assistant
I W NELSON, FRCS, clinical lecturer

Correspondence to: Mr A H R W Simpson, Nuffield Orthopaedic Centre, Oxford OX3 7LD.

Increased prevalence of non-secretors in patients with Graves' disease: evidence for an infective aetiology?

Graves' disease is associated with HLA-B8 and HLA-DR3 on chromosome 6. The abnormalities of humoral immunity causing the production of antibodies that stimulate thyroid stimulating hormone receptors, and the role of cell mediated immunity, are well known, but the trigger that allows predisposed people to develop the disease is not. Environmental influences such as stress and infection have been proposed.[1] An inability to secrete the water soluble glycoprotein form of the ABO blood group antigens is associated with increased susceptibility to several infections, particularly in children in the time between their losing maternal antibodies and developing their own active immunity. This characteristic does not alter with age or environment. The secretor gene is on chromosome 19 and is not linked to sex or the HLA markers. There is a significantly higher proportion of non-secretors in patients with insulin dependent diabetes mellitus[2] and ankylosing spondylitis[3] than in the general population. These conditions are closely associated with HLA markers, and an infective aetiology has been suggested for each. We examined the secretor state of patients with organ specific autoimmune thyroid disease. This group of diseases is not uncommon in patients with insulin dependent diabetes mellitus and their first degree relatives.

Patients, methods, and results

We studied 77 patients with Graves' disease, 33 with spontaneous primary atrophic hypothyroidism, and 26 with Hashimoto's thyroiditis. ABO and Lewis blood groups were determined by agglutination and the secretor state from saliva by the haemagglutination inhibition method.[4] Secretor state was confirmed by the Lewis blood group. The results were compared with those from local blood donors with the χ^2 test. The table shows that the proportion of non-secretors with Graves' disease was significantly increased compared with that in the control population ($p<0·005$). This was not true for patients with primary atrophic hypothyroidism or Hashimoto's thyroiditis. There was no significant difference in the distribution of ABO blood groups between any of the categories of thyroid disease and the control population.

Blood group and secretor state of patients with autoimmune thyroid disease and controls. Values are numbers (percentages) of subjects

	ABO blood group				Secretor state	
	A	B	O	AB	Secretor	Non-secretor
Graves' disease (n=77)	28 (36)	6 (8)	40 (52)	3 (4)	43 (56)	34 (44)
Spontaneous primary atrophic hypothyroidism (n=33)	11 (33)	3 (9)	18 (55)	1 (3)	23 (70)	10 (30)
Hashimoto's thyroiditis (n=26)	5 (19)	1 (4)	19 (73)	1 (4)	19 (73)	7 (27)
Controls (n=334)	104 (31)	42 (13)	173 (52)	15 (4)	245 (73)	89 (27)

Comment

Our results show that the inherited susceptibility to Graves' disease is associated with genetic markers on chromosome 19 as well as on chromosome 6. As some patients with Graves' disease eventually develop hypothyroidism it might have been expected that patients with primary atrophic hypothyroidism would show the same increased proportion of non-secretors. This was not so, but the number studied was small.

The increased susceptibility of non-secretors to infection raises the possibility that an infective agent may play a part in the development of Graves' disease. A striking association has been shown between the prevalence of antibodies against the enteric pathogen *Yersinia enterocolitica* and autoimmune thyroid disease, particularly in Scandinavia.[1] This organism, in addition to *Escherichia coli* and other Gram negative bacteria, has been shown to contain a binding site for thyroid stimulating hormone, and this is recognised by immunoglobulins from patients with Graves' disease.[1] Possibly the increased susceptibility of non-secretors to bacterial infection might result in the production of antibodies to microbial antigens that then cross react with the receptor for thyroid stimulating hormone and trigger the onset of hyperthyroidism.

1 Heyma P, Harrison LC, Robins-Browne R. Thyrotrophin (TSH) binding sites on Yersinia enterocolitica recognised by immunoglobulins from humans with Graves' disease. *Clin Exp Immunol* 1986;64:249-54.
2 Blackwell CC, James VS, Weir DM, *et al*. Secretor state of patients with insulin dependent or non-insulin dependent diabetes mellitus. *Br Med J* 1987;295:1024-5.
3 Shinebaum R, Blackwell CC, Forster PJG, *et al*. Non-secretion of ABO blood group antigens as a host susceptibility factor in the spondyloarthropathies. *Br Med J* 1987;294:208-10.
4 Mollison PL. *Blood transfusion in clinical medicine*. Oxford: Blackwell Scientific Publications, 1979:414-82.
5 Shenkman L, Bottone EJ. *Yersinia enterocolitica*. Florida: CRC Press, 1981:136-55.

(Accepted 18 January 1988)

University Department of Medicine, Royal Infirmary, Edinburgh EH3 9YW
A COLLIER, MRCP, medical registrar
A W PATRICK, MRCP, medical registrar
A D TOFT, MD, FRCPED, senior lecturer

University Department of Bacteriology, Medical School, Edinburgh EH8 9AG
C C BLACKWELL, PHD, lecturer
V JAMES, FIMLS, research technician
D M WEIR, FRCPED, professor

Correspondence to: Dr Toft.

Diabetes Research (1988) 9, 117–119

DIABETES RESEARCH 27
© TEVIOT-KIMPTON 1988

SECRETOR STATE AND COMPLEMENT LEVELS (C3 AND C4) IN INSULIN DEPENDENT DIABETES MELLITUS

C. C. BLACKWELL,[1] D. M. WEIR,[1] A. W. PATRICK,[2] A. COLLIER[2] and B. F. CLARKE[2]

[1]Department of Bacteriology, University of Edinburgh, The Medical School, Teviot Place, and [2]Diabetic and Dietetic Department, Royal Infirmary, Laurston Place, Edinburgh, UK

(Received 23 April 1988)

SUMMARY Lower levels of C3 and C4 components of the complement system have been reported for patients with insulin dependent diabetes mellitus (IDDM) but not among those with non-insulin dependent diabetes (NIDDM). We have found a significantly higher proportion of patients who are non-secretors of the ABO blood group antigens among patients with IDDM but not among those with NIDDM. As the gene that controls secretion of these antigens is in the same linkage group as that for the C3 complement component, we compared the levels of C3 and C4 of patients with IDDM by secretor state. The mean level of C3c for 45 non-secretors (75·2 IU/ml) was significantly lower than that found for 59 secretors (86·4 IU/ml) ($p < 0{\cdot}025$). The level of C4 among non-secretors (77·1 IU/ml) was also significantly lower than that of secretors (96·3 IU/ml) ($p < 0{\cdot}025$). The significance of these observations is discussed.

Key words: Secretor state, complement, C3, C4, insulin dependent diabetes mellitus

INTRODUCTION

THE C3 and C4 complement components play a central role in dealing with infectious agents and clearance of immune complexes. Lower levels of C3 and C4 have been found among patients with insulin dependent diabetes mellitus (IDDM) (1,2), but normal levels of these components are reported among patients with non-insulin dependent diabetes mellitus (NIDDM) (3). The gene for C3 is in the same linkage group as the gene that controls secretor status. In a previous study we found no significant differences in the mean levels of C3 for 109 secretors (126 IU/ml) and 110 non-secretors (120 IU/ml). There were, however, eight individuals with C3 levels below the lower limits of the normal range; seven of the eight were non-secretors (4). None of these eight donors was a diabetic.

Our recent survey found a significantly higher proportion of non-secretors among patients with IDDM (40·2%) but not among those with NIDDM (25·2%) compared with local controls (26·6%) (5). From this we predicted that the lower levels of C3 reported for patients with IDDM might be due to the increase in the proportion of non-secretors. To test this hypothesis we determined the C3 and C4 levels of patients with IDDM and compared these values by secretor status.

SUBJECTS AND METHODS

The study population consisted of patients attending routine diabetic clinics. They ranged in age from 18–65; there were 59 secretors and 45 non-secretors. None of the patients showed any signs of infection at the time of venepuncture.

Secretor state was determined from saliva by the haemagglutination inhibition method (6) and confirmed by Lewis blood group determined with polyclonal antisera (Behring). C3c and C4 levels were determined with Behring NOR-Partigen immunodiffusion plates. The results were recorded as international units (IU)/ml and the results compared by Student's t-test after conversion of the data to logarithms.

RESULTS

The table shows a significantly lower level of C3c among the non-secretors with IDDM (75·2 IU/ml) compared with the values for secretors (86·4 IU/ml) ($p < 0{\cdot}025$). The same pattern was observed for C4 values; the non-secretors (77·1 IU/ml) had significantly lower levels than

Correspondence: Prof. D. M. Weir, Department of Bacteriology, University of Edinburgh, The Medical School, Teviot Place, Edinburgh EH8 9AG, UK.

0265–5985/88/1100–0117 $05.50

Table 1. Comparison of the geometric means of C3c and C4 levels of secretors and non-secretors with IDDM

	secretors (59) (IU/ml)	*non-secretors (45)* (IU/ml)	*t*	*df*	*p*
C3c	86·4	75·2	2·03169	102	< 0·025
C4	96·3	77·1	2·10037	102	< 0·025

the secretors (96·3 IU/ml) ($p < 0·025$). The mean values for healthy adults given in the Table of Calibration Values for the NOR-partigen plates are 105 IU/ml for C3c and 96 IU/ml for C4. While the C3 value for secretors with IDDM is lower than the mean for the normal range, their C4 value for secretors is very close to the mean.

Discussion

The mean levels of C3 for both secretors (86·4 IU/ml) and non-secretor (75·2 IU/ml) patients in the present study were lower than those reported for central Europeans (105 IU/ml) and for secretors and non-secretors in our previous study, 126 IU/ml and 120 IU/ml respectively (4). Although immune complexes are found in a high proportion of patients with IDDM (7), the lower levels of C3 and C4 previously reported did not correlate with levels of complexes detected by C1 binding assays. Binding of these components to immune complexes was not excluded: but turnover studies suggested a major influence was reduction in synthesis of C3 and C4 (1).

The association between non-secretion of ABO antigens and reduced levels of C3 in IDDM remains unclear. It may be significant that the C3 gene is in the same linkage group as the secretor gene (8) which codes for fucosyl transferase. Prior to secretion complement components undergo post translational modifications that include glycosylation that influence intracellular catabolism of the products (9). Modification of normal glycosylation of complement components in IDDM may need to be considered.

The C4 gene, in contrast, is associated with the HLA region of chromosome 6 (10). The unexpected reduction in C4 levels in non-secretors suggests that reported effects of genes outside the MHC may be responsible. Deficiency in C4 production has been found in a patient with SLE and 6 healthy family members that was not linked to HLA, Bf or C4 structural loci (11). In studies in mice non-MHC genetic factors appear to be responsible for the post-translational modification of pro-C4 (12). Vergami *et al.* (13) suggested the lower levels of C4 in chronic active hepatitis patients and their relatives, may be due to non-MHC genetic influences such as post-translational modification.

In our earlier studies we have found significantly higher proportions of non-secretors among patients with recurrent urinary tract infections (13–15) and superficial candida infections (16, 17), two common infectious problems encountered among diabetics. A higher proportion of non-secretors has been found among "normal" individuals colonized by *Candida* species (18) and uropathogenic *Escherichia coli* strains bind in greater numbers to cells of non-secretors (19).

An infectious trigger has been postulated for IDDM (20). We have suggested that the non-immune non-secretor host might be more susceptible to the original colonization by infectious agents (4). The specific immune responses to the agent controlled by the class II HLA DR3-4 genes and the lower levels of C3 and C4 might contribute to the pathogenic sequelae that result in the diabetic state.

Acknowledgements

We are grateful to Dr. A. E. Mourant for helpful discussions and to the Edinburgh and Southeast Scotland Blood Transfusion Service for reagents used in the study. This work was supported in part by a grant from the Wellcome Trust.

References

1. Charlesworth, J. A., Timmermanns, V., Golding, J., *et al.* (1987). The complement system in type I (insulin dependent) diabetes. *Diabetologia*, **30**, 372–379.
2. Vergami, D., Johnston, C., B-Abdullah, N. and Barnet, A. H. (1983). Low serum C4 concentrations: an inherited predisposition to insulin dependent diabetes. *Brit. Med. J.*, **286**, 926–928.
3. Charlesworth, J. A., Campbell, L. V., Catanzaro, R., *et al.* (1982). Immune complexes in diabetes mellitus: studies of complement utilization and tissue deposition. *J. Clin. Lab. Immunol.*, **8**, 163–168.
4. Blackwell, C. C., Weir, D. M., James, V. S., *et al.* (1988). Non-secretion of ABO blood group antigens and susceptibility to infectious diseases. NATO Advanced Workshop—Complement, Phagocytosis and Bacteria (in press).
5. Blackwell, C. C., James, V. S., *et al.* (1987). Secretor state of patients with insulin dependent or non-insulin dependent diabetes mellitus. *Brit. Med. J.*, **295**, 1024–1025.
6. Mollinson, P. L. (1979). *Blood Transfusion in Clinical Medicine*, pp. 414–482. Blackwell Scientific Publications, Oxford.
7. Di Mario, U., Lavicoli, M. and Andreani, D. (1980). Circulating immune complexes in diabetes. *Diabetologia*, **19**, 89–92.
8. Whitehead, A. S., Solomon, E., Chambers, S. P., *et al.* (1982). Assignment of the gene for the third component of complement (C3) to chromosome 19. *Proc. Natl. Acad. Sci. U.S.A.*, **79**, 5021–5025.
9. Matthews, J., Goldberger, G., Marino, J. T., *et al.* (1982). Complement proteins C2, C4 and factor B. Effect of glycosylation on their secretion and catabolism. *Biochem. J.*, **204**, 839–846.
10. Alper, C. A. (1981). In *The Role of the Major Histocompatibility Complex in Immunobiology* (edited by M. E. Dorf), pp. 173–220. Garland Press, New York.
11. Muir, W. A., Hedrick, S., Alper, C. A., *et al.* (1984). Inherited incomplete deficiency of the fourth component of complement (C4) determine by a gene not linked to human histocompatibility leucocyte antigens. *J. Clin. Invest.*, **74**, 1509–1514.
12. Karp, D. R., Atkinson, J. P. and Shreffler, D. C. (1982). Genetic variation in glycosylation of 4th component of murine complement. *J. Biol. Chem.*, **257**, 7330–7335.
13. Vergami, D., Wells, L., Larcher, V. F., *et al.* (1985). Genetically determined low C4: a predisposing factor to autoimmune chronic active hepatitis. *Lancet*, ii, 294–298.

14. Blackwell, C. C., May, S. J., Brettle, R. P., *et al.* (1986). Host-parasite interactions underlying non-secretion of blood group antigens and susceptibility to recurrent urinary tract infections. In *Protein-Carbohydrate Interactions in Biological Systems* (edited by D. Lark), pp. 229–230. Academic Press, London.
15. Blackwell, C. C., May, S. J., Brettle, R. P., *et al.* (1987). Secretor state and immunoglobulin levels among women with recurrent urinary tract infections. *J. Clin. Lab. Immunol.*, **22**, 133–137.
16. Blackwell, C. C., Thom, S. M., Lawrie, O. R., *et al.* (1986). Non-secretion of blood group antigens and susceptibility to oral infections by *Candida albicans*. *J. Dent. Res.*, **65**, 502.
17. Thom, S. M., Blackwell, C. C., Weir, D. M. *et al.* (1989). Non-secretion of blood group antigens and susceptibility to infection by *Candida* species. *FEMS Microbiol. Immunol.*, (in press).
18. Burford-Mason, A. P., Weber, J. C. P. and Willoughby, J. M. T. (1988). Oral carriage of *Candida albicans*, ABO blood group and secretor status in healthy individuals. *J. Med. Vet. Mycology*, **26**, 49–56.
19. Lomberg, H., Cedergren, B., Leffler, H., Nilsson, B., *et al.* (1986). Influence of blood group on the availability of receptors for attachment of uropathogenic *Escherichia coli*. *Infect. Immun.*, **51**, 919–926.
20. Botazzo, G. F. (1986). Death of a beta cell: homicide or suicide? *Diabet. Med.*, **3**, 119–130.

Leading Article

GENETIC SUSCEPTIBILITY TO INFECTIOUS AGENTS

C. Caroline Blackwell, Department of Bacteriology, University Medical School, Edinburgh

> 'Much more attention should be given to the combined effects of blood group and secretor status on susceptibility to bacterial infection.' A.E. Mourant, FRS.[1]

It is appropriate that this article is published in the April issue of the *Proceedings* as Dr Arthur Mourant will be 85 on the 11th of the month. As he pointed out in his introductory chapter to *Blood Groups and Diseases*, associations between these genetic markers and human diseases had long been of interest. It was, however, only in 1953 that the first convincing evidence was found, an association between group A and carcinoma of the stomach.[2] The group led by C.A. Clarke and R.B. McConnell at Liverpool found additional associations of other forms of cancer and ulcers with ABO blood groups and with the ABH secretor system.[3]

The ABH secretor system has been of interest mainly to two very different groups: physical anthropologists who used blood groups and secretor status to examine genetic markers of different races; and blood transfusion services because it affects expression of the Lewis blood group antigens (Table 1). The ability to secrete the ABO blood group antigens into body fluids is controlled by a single gene on chromosome 19. As it is inherited in a dominant pattern the predicted proportion of secretors within a population is 75 per cent and that for the recessive non-secretor phenotype 25 per cent. This ratio can vary widely in different ethnic groups and some geographically isolated populations.[3,4] When *Blood Groups and Diseases* was published in 1978, nearly 25 pages were devoted to the reported studies of associations of ABO blood groups with infectious diseases. Less than one page summarized the studies on secretor status and infection.

EPIDEMIOLOGICAL STUDIES

Infectious diseases

Since the early 1980s work in the Department of Bacteriology at Edinburgh

TABLE 1

Blood group antigens found in the body fluids of secretors and non-secretors.

	Blood group antigens in body fluids				
	*H**	*A†*	*B*	*Lewis[a]*	*Lewis[b]*
Secretors	+	+/0	+/0	(±)	+
Non-secretors	0	0	0	+	0

*H is the antigen of blood group O.
†Depending on the presence of the A and/or B gene.

TABLE 2

Associations between non-secretion and susceptibility to infection.

Infectious agent	*Increased proportion of non-secretors*	*Reference*
URINARY TRACT		
Esch. coli	Yes	
adults		5, 6
children		7
GASTROINTESTINAL TRACT		
V. cholerae	Yes	8
GENITAL TRACT		
C. albicans	Yes	9, 10
C. trachomatis	Yes	Blackwell (unpublished observations)
N. gonorrhoeae	No	11, 12
RESPIRATORY TRACT		
Strep. pyogenes (carriage and rheumatic fever)	Yes	13
N. meningitidis	Yes	14
Strep. pneumoniae	Yes	14
H. influenza type b	Yes	15
ORAL CAVITY		
C. albicans infections	Yes	10, 16
C. albicans carriage	Yes	17
Periodontal disease	No	18
Caries	Yes	19, 20

University has expanded the studies on secretor status and susceptibility to infectious diseases (Table 2). These surveys were made possible through the cooperative efforts of colleagues from a variety of clinical disciplines in Britain, Iceland and Nigeria.

Studies of secretor status among patients with meningococcal disease and their local controls (Table 3) suggested that in areas of the world where there were prolonged outbreaks or epidemics (Iceland and Nigeria), there was an unusually high proportion of non-secretors in the indigenous population. During the prolonged outbreak of meningococcal disease that occurred in Gloucestershire from 1984–1987, the town of Stonehouse had an attack rate at least 10 times greater than other parts of the county. In November 1986 our research team participated in the Stonehouse Study, a project in which over 6,000 residents and children attending school in Stonehouse were screened for carriage of the outbreak strain, antibody to the outbreak strain and secretor status.[23]

As we predicted, there was a significantly higher proportion of non-secretors in Stonehouse, 33 per cent compared with 23 per cent for the blood donors in the Southwestern Region ($P<0{\cdot}005$). This cannot account in itself for the outbreak, but it indicates there is a significantly larger proportion of individuals genetically susceptible to infection in the town.[24]

Autoimmune diseases

Associations between genes of the major histocompatibility complex (MHC) and

TABLE 3
Secretor status of patients with meningococcal infection.

		Non-secretors		
	Total	*No.*	*Per cent*	*P*
Scotland				
Controls[5]	334	89	26·6	<0·005
Patients[14]	26	18	69	
Stonehouse, England				
Controls[24]	4906	1352	27·6	
Patients[24]	13	7	54	
Iceland				
Controls[21]	228	92	41·2	<0·05
Patients[14]	98	53	54	
Nigeria				
Controls[22]	186	92	49·5	<0·01
Patients[22]	42	31	73·3	

TABLE 4
Association between secretor status and susceptibility to autoimmune diseases.

Disease	*Increased proportion of non-secretors*	*Reference*
RHEUMATIC DISEASES		
Rheumatic fever	Yes	13
Ankylosing spondylitis	Yes	26
Rheumatoid arthritis	No	27
Psoriasis	No	27
Psoriatic arthropathy	Yes	27
ENDOCRINE DISEASES		
Diabetes mellitus, unclassified	Yes	30
insulin dependent	Yes	28
non-insulin dependent	No	28
Hashimoto's thyroiditis	No	29
Primary atrophic hypothyroidism	No	29
Graves' disease	Yes	29

several autoimmune diseases have been established,[25] but the existence of additional genetic susceptibility factors outside the MHC cluster on chromosome 6 was predicted. Our studies on infectious diseases suggested there might be an association between non-secretion and some autoimmune diseases for which infectious aetiologies have been proposed; and this is the pattern we found among patients with several rheumatic diseases, insulin dependent diabetes (IDDM) and Graves' disease.[26–29] (Table 4).

A review of available data on HLA markers associated with IDDM and secretor status of ethnic groups in which the disease is rare was striking. IDDM is rarely found among American Indians and Eskimos.[31] They do not lack the HLA

TABLE 5

Frequency of HLA B8, DR3 and DR4 and proportion of non-secretors in Caucasian and Indian populations[25,32].

Ethnic group	Frequencies of B8	DR3	DR4	Per cent non-secretors
American Caucasians	17·1	22·2	27·3	20–25
American Indians	2·9	6	47·8	0–2

TABLE 6

Proportion of HLA B27 and non-secretors in different ethnic groups[32,33,35].

Ethnic group	Per cent B27	Per cent non-secretors
Haida	50	25
Eskimo	20–30	0–2
Lapps	20–30	2–5
Caucasians	8	20–25

antigens associated with IDDM,[25] but, compared with Caucasians, non-secretors are very much a minority (0–2%) (Table 5).[32]

The Haida Indians of British Columbia have an unusually high prevalence of ankylosing spondylitis (10% among adult males) and the associated HLA B27 marker (50%).[33] In addition, the proportion of non-secretors (25%) among the Haidas is similar to that of Caucasians, at least ten-fold greater than that reported for other Canadian Indians.[32] Although 20–30% of American Indians, Eskimos and Lapps are B27 positive, the proportion of non-secretors in each of these groups is very low, and the prevalence of ankylosing spondylitis is lower among these groups than among the Haida (Table 6).[33]

Susceptibility of non-secretors to infectious agents

Although the ABO blood groups and secretor status are stable, easily and inexpensively determined genetic markers, investigation of these early epidemiological observations was not actively pursued for many years. There are probably a number of reasons for this. Different research groups obtained different ABO blood group associations for the same diseases (Table 7). These discrepancies might be due to variation in the strains causing the outbreaks studied,[44–46] or there might be differences in populations or patient categories examined.[11,12,41,42] With the advent of tissue and organ transplantation, advances in definition of the MHC overshadowed studies of blood group antigens with reference to immunity and infection. In my opinion however, the main reason these observations were not pursued is that only one hypothesis was suggested to

TABLE 7

Associations between ABO blood groups and susceptibility to infection.

Infectious agent/disease	*Blood group association*	*Reference*
URINARY TRACT		
Esch. coli	B	36
	B/AB	5
GASTROINTESTINAL TRACT		
Esch. coli	B	37
Salmonella and *Esch. coli*	B and AB	38
V. cholerae	O	39, 40
GENITOURINARY TRACT		
N. gonorrhoeae	B	11, 41, 42
	No association	12
C. trachomatis	B	Blackwell (unpublished results)
RESPIRATORY TRACT		
Strept. pyogenes Group A	not O	13
Strept. pneumoniae	not B	43
Influenza A_2	O	44
Influenza A	O	45
	B	46
M. tuberculosis	O	47
ORAL CAVITY		
Periodontal disease	O and AB	18
C. albicans (carriage)	O	17
BLOOD BORNE INFECTIONS		
Malaria	A	48
Coccidioides immitis	B	49

explain these associations. Many microorganisms possess antigens crossreactive with the ABO antigens[42,50,51] or incorporate blood group antigens into their cell envelopes.[43] Anti-A and anti-B isohaemagglutinins might act as 'natural antibodies' against strains with these surface components in a non-immune host.

Since the mid-1970s it has become increasingly apparent that protein–carbohydrate interactions play an important role in the phagocytosis of microorganisms in the non-immune host,[52,53] and many adhesins on microorganisms bind preferentially to carbohydrate receptors on epithelial cells.[54] Although there is no direct evidence for ABO or Lewis antigens acting as host cell receptors, several pathogens bind to other blood group antigens (Table 8). These findings have led to some new hypotheses outlined below.

Reduction of colonisation. If microbial adhesins bind to ABO or Lewis blood groups on epithelial cells, the secreted forms of these antigens might interfere with attachment of the microorganisms to the mucosa. Attachment of *Strept. salivarius* to epithelial cells is inhibited by salivary glycoproteins with blood group activity,[62] and heat-treated saliva from secretors reduces binding of *Candida* to epithelial cells.[9,10] These interactions might contribute to the increased proporton of non-secretors among carriers of group A *Strept. pyogenes*[13] or *C. albicans*.[17]

TABLE 8

Blood group antigens that are receptors for microorganisms.

Microorganism	*Blood group receptor*	*Reference*
Esch. coli		
Uropathogenic strains	P	55
	M	56
	N	57
Septicaemia and neonatal meningitis	S	58
H. influenzae type B	Anton	59
Candida	Lewis[a]	60
Plasmodium knowlesi	Duffy	61

Lewis[a] acts as a receptor for some strains of microorganisms. Uropathogenic strains of *Esch. coli* bind in greater numbers to cells from non-secretors than to cells from secretors.[63] A major difference in the antigens on the mucosa of non-secretors is the presence of Lewis[a]. Binding of one candida strain was reduced if non-secretor cells were pre-treated with anti-Lewis[a] antisera, but treatment of secretor cells with anti-Lewis[b] did not reduce binding.[60]

Alteration of a receptor by the product of the secretor gene. The product of the secretor gene is a fucosyl transferase. It has been suggested that this enzyme might alter carbohydrate antigens on the cell surface, reducing access of the microorganism to its receptor.[63]

Immune responses of non-secretors. Lower levels of both salivary and serum IgA are reported for non-secretors;[64,65] however, our studies found neither salivary nor serum IgA responses of non-secretors to be impaired. In most patient groups, the non-secretors had higher levels of total serum IgA. The differences in total IgA levels for secretors and non-secretors observed in these groups require further investigation for their possible roles in protection of mucosal surfaces, or conversely as non-complement fixing/blocking antibodies in the pathogenesis of infectious diseases and their sequelae.[6,66]

The complement system of non-secretors. Most of the genes for the complement system are on chromosome 6, but the structural gene for the third component of the system (C3) is in the same linkage group as the secretor gene. Although C3 levels of 100 non-secretors in the Stonehouse survey were not significantly lower than 100 age-matched secretors, 7 of the 8 individuals whose C3 levels were below the lower limits of the normal range were non-secretors.[67] Among diabetic patients C3 and C4 levels are lower in those with IDDM than in those with NIDDM or in non-diabetic individuals.[68] Among patients with IDDM, we found levels of C3 and C4 significantly lower among non-secretors compared with secretors.[69]

Selective advantage of non-secretion. If non-secretors are more susceptible to infectious agents, why does the phenotype persist? Mourant and others have suggested that ABO incompatibility of the fetus with respect to the mother

determines ABO haemolytic disease of the newborn and also early spontaneous abortions. Among infants with ABO haemolytic disease there is a marked deficiency of non-secretors. A considerable proportion of aborted fetuses are anatomically normal, but show various degrees of necrosis. This has been suggested to result from attack by the maternal antibodies on incompatible blood group antigens in fetal tissues. There is a selective loss of group A and B fetuses and neonates and this is partly controlled by the secretor gene.[70]

CONCLUSIONS

The ability to secrete the ABO blood group antigens appears to play a role in protecting individuals whose specific immune defences are compromised in some way. The compromising factors range from absence of protective antibodies to meningococci in young children to less well defined reduced T cell functions observed during pregnancy. Future investigations should include examination of the role of secretor status in development of opportunistic infections among patients who are immunosuppressed following chemotherapy or organ transplantation or those infected with the human immunodeficiency virus (HIV).

Identification of non-secretion as a susceptibility factor might be of predictive value in the study of AIDS. Because HIV infection in male homosexuals and intravenous drug users bypasses the defence of skin and mucosa, the proportion of secretors and non-secretors in these two groups probably reflects that of the local uninfected population. The role of secretor status in heterosexual transmission of HIV and in development of opportunistic infections during the course of the disease remains to be determined. If secretion plays a role in the innate host defences, as the specific immune function declines, non-secretors might be susceptible to organisms such as *Candida* earlier in the course of the disease than secretors.

Identification of the associations of non-secretion with susceptibility to particular infectious and autoimmune diseases provides an additional genetic marker that can be used in epidemiological studies and in investigation of the complex interactions that lead to these disease states. It also suggests that in light of recent studies on the role of *Campylobacter pylori* in the aetiology of peptic ulcers, the old associations of non-secretion with ulcers and with some malignancies might be reviewed with reference to infectious aetiologies.

ACKNOWLEDGEMENTS

I am grateful to Professor D.M. Weir for his critical reading of the manuscript and to Mrs M. Cole for help in its preparation.

FURTHER READING

In 1989 a special issue of FEMS Microbiology Immunology has been devoted to the association of blood groups and secretor status with diseases to celebrate Dr Mourant's 85th birthday and to recognize his contribution to initiation of these studies.

REFERENCES

[1] Mourant AE. Blood relations, blood groups and anthropology. Oxford: Oxford University Press 1983.

[2] Aird I, Bentall HH, Roberts JA. A relationship between cancer of the stomach and the ABO blood groups. *Brit Med J* 1953; i: 799–801.

[3] Mourant AE, Kopeć AC, Domaniewska-Sobczak K. Blood groups and diseases. Oxford: Oxford University Press 1978.

[4] Erikson AW, Partanen K, Frants RR, Pronk JC, Kostense PJ. ABH secretion polymorphism in Icelanders: Åland islanders, Finns, Finnish Lapps, Komi and Greenland Eskimos—a review and new data. *Annal Hum Biol* 1986; **13:** 273–285.

[5] Kinane DF, Blackwell CC, Brettle RP *et al.* ABO blood group, secretor state and susceptibility to recurrent urinary tract infection in women. *Br Med J* 1982; **285:** 7–9.

[6] Blackwell CC, May SJ, Brettle RP *et al.* Secretor state and immunoglobulin levels among women with recurrent urinary tract infections. *J Clin Lab Immunol* 1987; **22:** 133–137.

[7] Lomberg H, Hellstrom M, Jodal U *et al.* Secretor state and renal scarring in girls with recurrent pyelonephritis. *FEMS Microbiol Immunol* (in press).

[8] Chaudhuri A, Das Adhikary CR. Possible role of blood group secretory substances in the aetiology of cholera. *Trans Roy Soc Trop Med Hyg* 1978; **72:** 664–665.

[9] Blackwell CC, Thom SM, Weir DM *et al.* Host-parasite interactions underlying non-secretion of blood group antigens and susceptibility to infections by *Candida albicans*. In: Protein–carbohydrate interactions in biological systems. Lark D (ed). London: Academic Press 1986.

[10] Thom SM, Blackwell CC, MacCallum CJ *et al.* Non-secretion of ABO blood group antigens and susceptibility to infection by *Candida* species. *FEMS Microbiol Immunol* [in press].

[11] Kinane DF, Blackwell CC, Winstanley FP *et al.* Blood group, secretor status and susceptibility to infection by *Neisseria gonorrhoeae*. *Br J Vener Dis* 1983; **59:** 44–46.

[12] Johnson AP, Osborn MF, Hanna NF *et al.* A study of the relationship between ABO blood groups, secretor status and infection with *Neisseria gonorrhoeae*. *J Infect* 1983; **6:** 171–174.

[13] Haverkorn MJ, Goslings WRO. Streptococci, ABO blood groups and secretor status. *Am J Hum Genet* 1969; **21:** 360–375.

[14] Blackwell CC, Jónsdóttir K, Hanson M *et al.* Non-secretion of ABO blood group antigens predisposing to infection by *Neisseria meningitidis* and *Streptococcus pneumoniae*. *Lancet* 1986; **ii:** 284–285.

[15] Blackwell CC, Jónsdóttir K, Hanson MF *et al.* Non-secretion of ABO blood group antigens predisposing to infection by *Haemophilus influenzae*. *Lancet* 1986; **ii:** 687.

[16] Blackwell CC, Thom SM, Lawrie O *et al.* Non-secretion of blood antigens and susceptibility to oral infections due to *Candida albicans*. *J Dent Res* 1986; **65:** 502.

[17] Burford-Mason AP, Weber JCP, Willoughby JMT. Oral carriage of *Candida albicans*, ABO blood group and secretor status in healthy individuals. *J Med Vet Mycology* 1988; **26:** 49–56.

[18] Pradhan AC, Chawla TN, Samuel KC *et al.* The relationship between peridontal disease and blood groups and secretor status. *J Perio Res* 1971; **6:** 294–300.

[19] Arneberg P, Kornstad L, Nordbo H *et al.* Less dental caries among secretors than among non-secretors of blood group substance. *Scand J Dent Res* 1976; **84:** 362–366.

[20] Holbrook WP, Blackwell CC. Secretor state and dental caries in Iceland. *FEMS Microbiol Immunol* (in press).

[21] Thordarson G, Bjarnason C, Lincoln PJ *et al.* ABH secretor and Lewis type frequencies in an Icelandic Series. *J Med Genet* 1972; **9:** 46–47.

[22] Blackwell CC, Jónsdóttir K, Mohammed I *et al.* Non-secretion of blood group antigens: a genetic factor predisposing to infection by *Neisseria Meningitidis*. In: Gonococci and Meningococci. Poolman JT *et al.* (eds). Dordrecht: Kluwer Academic Publishers 1988.

[23] Stuart JM, Cartwright KAV, Jones DM *et al.* An outbreak of meningococcal disease in Stonehouse: planning and execution of a large scale study. *Epidemiol Inf* 1987; **99:** 579–589.

[24] Blackwell CC, Weir DM, James VS *et al.* The Stonehouse study: secretor status and carriage of *Neisseria* species. *Epidemiol Inf* 1989; **102:** 1–10.

[25] Tiwari JL, Terasaki PI. HLA and disease associations. New York: Springer-Verlag 1985.

[26] Shinebaum R, Blackwell CC, Forster PJG *et al.* Non-secretion of ABO blood group antigens as a host susceptibility factor in the spondylarthropathies. *Br Med J* 1987; **294:** 208–210.

[27] Shinebaum R, Blackwell CC, Abernethy VE *et al.* Secretor state of patients with psoriasis, psoriatic arthropathy or rheumatoid arthritis. *Br J Rheum Dis* 1987; **76** (Suppl 2): 105.

[28] Blackwell CC, James VS, Weir DM *et al.* Secretor state of patients with insulin dependent or non-insulin dependent diabetes mellitus. *Br Med J* 1987; **295**: 1024–1025.
[29] Collier A, Patrick AW, Toft AD *et al.* Increased prevalence of non-secretors in patients with Graves disease—evidence for an infective aetiology? *Br Med J* 1988; **296**: 1162.
[30] Andersen J, Lauritzen E. Blood groups and diabetes mellitus. *Diabetes* 1960; **9**: 20–24.
[31] Skyler JS. Insulin-dependent diabetes mellitus. In Clinical Endocrinology. Kohler PO, Jordon RM (eds). New York: John Wiley and Sons 1986.
[32] Mourant AE, Kopeć AC, Domaniewska-Sobczak K. The distribution of the human blood groups and other polymorphisms. London: Oxford University Press 1976.
[33] Gofton JP, Chalmers A, Price GE *et al.* HLA 27 and ankylosing spondylitis in B.C. Indians. *J Rheumatol* 1975; **2**: 314–318.
[34] Blackwell CC, Weir DM. HLA markers, secretor status and autoimmune diseases in different ethnic groups (submitted for publication).
[35] Dausset J, Colombani J. Histocompatibility testing. Copenhagen: Monksgaard 1973.
[36] Cruz-Coke R, Paredes L, Montenegro A. Blood groups and urinary microorganisms. *J Med Genet* 1965; **2**: 185–188.
[37] Socha W, Bilinska M, Kaczera Z. *Escherichia coli* and ABO blood groups. *Folia Biol (Krakola)* 1969; **17**: 259–269.
[38] Robinson MG, Tolchin D, Halpern C. Enteric bacterial agents and the ABO blood groups. *Am J Hum Genet* 1971; **23**: 135–145.
[39] Barua D, Paguio AS. ABO blood groups and cholera. *Ann Hum Biol* 1977; **4**: 489–492.
[40] Chaudhuri A, De S. Cholera and blood groups. *Lancet* 1977; **ii**: 404.
[41] Foster MT, Labrum AH. Relation of infection with *Neisseria gonorrhoeae* to ABO blood groups. *J Infect Dis* 1976; **133**: 329–330.
[42] Miler JJ, Novotny P, Walker PD *et al.* *Neisseria gonorrhoeae* and ABO isohaemagglutinins. *Infect Immun* 1977; **15**: 713–719.
[43] Reed WP, Drach GW, Williams RC Jr. Antigens common to human and bacterial cells. IV. Studies of human pneumococcal disease. *J Lab Clin Med* 1974; **83**: 599–610.
[44] McDonald JC, Zuckerman AJ. ABO blood groups and acute respiratory virus disease. *Br Med J* 1962; **ii**: 89–90.
[45] Potter CW. Haemagglutination inhibition antibody to various influenza viruses and adenoviruses in individuals of blood groups A and O. *J Hyg (Camb)* 1969; **67**: 67–74.
[46] Mackenzie JS, Fimmell PJ. The effect of ABO blood groups on the incidence of epidemic influenza and on the response to live attenuated and detergent split influenza vaccines. *J Hyg (Camb)* 1978; **80**: 21–30.
[47] Viskum K. The ABO Rhesus blood groups in patients with pulmonary tuberculosis. *Tubercle* 1975; **56**: 329–334.
[48] Gupta M, Chowdhuri ANR. Relationship between ABO blood groups and malaria. *Bull WHO* 1980; **58**: 913–915.
[49] Derensiski SC, Papagianis D, Stevens DA. Association of ABO blood groups and outcome of coccidioidal infection. *Sabouraudia* 1979; **17**: 261–264.
[50] Springer GF, Williamson P, Brandes WC. Blood group activity of gram-negative bacteria. *J Exp Med* 1961; **113**: 1077–1093.
[51] Landau JW, Fujiwara A, Newcome V *et al.* The relationship of commonly encountered saprophytic and pathogenic fungi to human A and B blood group antigens. *J Invest Dermatol* 1964; **43**: 59–70.
[52] Weir DM. Surface carbohydrates and lectins in cellular recognition. *Immunol Today* 1980; **2**: 45–51.
[53] Weir DM. Carbohydrates as recognition molecules in infection and immunity. *FEMS Microbiol Immunol* (in press).
[54] Lark D. Protein-carbohydrate interactions in biological systems. London: Academic Press 1986.
[55] Kallenius G, Mollby R, Svensson SB *et al.* The pk antigen as receptor for the haemagglutinin of pyelonephritic *Escherichia coli*. *FEMS Microbiol Lett* 1980; **7**: 297–302.
[56] Vaisanen V, Korhonen TK, Jokinen M *et al.* Blood group M specific haemagglutinin in pyelonephritic *Escherichia coli*. *Lancet* 1982; **i**: 1192.
[57] Jann K, Hoschutzky H, Mach T. Chemical nature and cellular location of adhesins of *E. coli*. In:

Bacteria, complement and the phagocytic cell. Cabello FC, Pruzzo C (eds) ASI series vol. 24. Berlin Springer-Verlag 1988.

[58] Korhonen TK, Vaisanen-Rhen V, Rhen M *et al. Escherichia coli* fimbrial recognizing sialyl galactosides. *J Bact* 1984; **159**: 762–766.

[59] Van Alphen L, Poole J, Overbeeke M. The Anton blood group antigen is the erythrocyte receptor for *Haemophilus influenzae*. *FEMS Microbiol Lett* 1986; **37**: 69–71.

[60] May SJ, Blackwell CC, Weir DM. Non-secretion of blood group antigens and susceptibility to *Candida albicans*: the role of Lewis blood group antigens. *J Dent Res* 1986; **65**: 503.

[61] Miller LH, Mason SJ, Dvorak JA *et al.* Erythrocyte receptors for (*Plasmodium knowlesi*) malaria. Duffy blood group determinants. *Science* 1975; **189**: 561–563.

[62] Williams RC, Gibbons RJ. Inhibition of streptococcal attachment to receptors on human buccal epithelial cells by antigenically similar salivary glycoproteins. *Infect Immun* 1975; **11**: 711–718.

[63] Lomberg H, Cedergren B, Leffler H *et al.* Influence of blood group on the availability of receptors for attachment of uropathogenic *Escherichia coli*. *Infect Immun* 1986; **51**: 919–926.

[64] Waissbluth JG, Langman JS. ABO blood groups, secretor status, salivary protein, and serum and salivary immunoglobulin concentrations. *Gut* 1971; **12**: 646–649.

[65] Grundbacher FJ. Immunoglobulins, secretor status and the incidence of rheumatic fever and rheumatic heart disease. *Hum Hered* 1972; **25**: 399–404.

[66] Blackwell, CC, Jonsdottir K, Hanson MF *et al.* Blood group, secretor status and susceptibility to bacterial meningitis. *FEMS Microbiol Immunol* (in press).

[67] Blackwell CC. The role of ABO blood groups and secretor status in host defences. *FEMS Microbiol Immunol* [in press].

[68] Charlesworth JA, Timmermans V, Golding J *et al.* The complement system in type 1 (insulin-dependent) diabetes. *Diabetologia* 1987; **30**: 372–379.

[69] Blackwell CC, Weir DM, Patrick AW *et al.* Secretor state and complement levels (C3 and C4) in insulin dependent diabetes mellitus. *Diabetes Research* (in press).

[70] Mourant AE. ABH secretion and natural selection. *Ann Hum Biol* 1982; **9**: 575–577.

Epidem. Inf. (1989), **102**, 1–10
Printed in Great Britain

The Stonehouse study: secretor status and carriage of *Neisseria* species

C. C. BLACKWELL,[1] D. M. WEIR,[1] V. S. JAMES,[1] K. A. V. CARTWRIGHT,[2] J. M. STUART[3] AND D. M. JONES[4]

[1] *Department of Bacteriology, University of Edinburgh* [2] *Public Health Laboratory, Gloucester GL1 3NH* [3] *Department of Community Medicine, Gloucester GL1 1LY* [4] *Public Health Laboratory, Manchester M20 8LR*

SUMMARY

The genetically determined inability to secrete the water-soluble glycoprotein form of the ABO blood group antigens into saliva and other body fluids is a recognized risk factor for meningococcal disease. During a community-wide investigation of a prolonged outbreak of disease due to a B15:P1.16 sulphonamide-resistant strain of *Neisseria meningitidis* in Stonehouse, Gloucestershire (the Stonehouse survey), the ABO blood group and secretor status of almost 5000 residents was determined.

The proportion of non-secretors in the Stonehouse population was significantly higher than the proportion of non-secretors among blood donors in the South West Region and in England generally. Seven of 13 Stonehouse residents with meningococcal disease who were tested were found to be non-secretors, a high proportion. The outbreak in Stonehouse cannot be explained solely in terms of the increased proportion of non-secretors. There was no clear correlation between the proportions of non-secretors in different areas within the town and the incidence of cases of meningococcal disease.

Carriers of meningococci, whether outbreak or other strains, were not more likely to be non-secretors. The reasons why non-secretors are more susceptible to meningococcal disease remain to be determined, but they do not appear to be related to carriage of meningococci.

INTRODUCTION

The large-scale survey undertaken in Stonehouse, Gloucestershire provided an opportunity to examine factors that might explain the prolonged local outbreak of disease due to serogroup B, serotype 15 P1.16, sulphonamide-resistant (B15 P1.16R) *Neisseria meningitidis* (Stuart *et al.* 1987). The results of the bacterial screening established that there was a low carriage rate of the B15 P1.16R strain in a defined community at a time of high disease activity. An outbreak continuing for several years implies that a virulent strain is moving slowly through a susceptible community. The factors responsible for the apparent virulence of the outbreak strain remain to be elucidated (Cartwright *et al.* 1987).

Environmental influences contributing to susceptibility of the host to these infections include severe overcrowding (Kaiser *et al.* 1974) and passive smoking

(Haneberg *et al.* 1983; Stuart *et al.* 1988). The former does not appear to play a significant role in the Stonehouse outbreak. Cigarette consumption is higher among men and women in unskilled jobs compared with those in professional employment (Wald *et al.* 1988), and there was a higher proportion of household heads with manual occupations in the two areas of the town with 14 of the 15 cases (Stuart *et al.* 1987).

We have identified a genetic character that is found in a significant proportion of patients with bacterial meningitis compared with individuals within their respective local control populations. This is the inability of an individual to secrete the water-soluble glycoprotein form of ABO blood group antigens (Blackwell *et al.* 1986*a*, *b*). The secretor gene is inherited in a Mendelian-dominant pattern; in most west European populations 20–25% express the recessive non-secretor phenotype associated with increased susceptibility to meningococcal infections (Eriksson *et al.* 1986; Race & Sanger, 1975). In areas of the world in which there have been prolonged outbreaks or epidemics of meningococcal disease such as Iceland and northern Nigeria and secretor status has been investigated, the proportions of non-secretors in these populations are higher. In Iceland non-secretors make up about 40% of the population (Thordarson *et al.* 1972), and near Maiduguri, Nigeria almost 50% (Blackwell *et al.* 1988). One objective of the survey was, therefore, to determine the proportion of non-secretors in Stonehouse.

There is a significantly higher proportion of non-secretors among patients with rheumatic fever and also a higher proportion of non-secretors among carriers of group A *Streptococcus pyogenes* (Haverkorn & Gosling, 1969). The increased susceptibility of non-secretors to meningococcal disease might be due to an increased tendency to acquire the outbreak strain compared with secretors. The second objective was to establish the proportion of non-secretors amongst carriers of meningococci, especially those carrying the outbreak strain.

SUBJECTS AND METHODS

The population examined has been described in detail (Stuart *et al.* 1987). ABO blood groups were determined for Stonehouse residents who participated in the study and from whom a blood sample (EDTA anti-coagulant tube) was collected. Because contamination of saliva with blood can result in a false positive secretor result when tested by the haemagglutination inhibition method, every eighth specimen was tested for Lewis blood group. Secretors express Lewis^b on their erythrocytes and non-secretors Lewis^a. Generally, 3–4% of individuals express neither Lewis^a nor Lewis^b on their erythrocytes; the majority of these are secretors by the saliva test. ABO group was determined by slide agglutination with monoclonal anti-A and anti-B antibodies supplied by the Edinburgh and South East Scotland Blood Transfusion Service. Lewis groups were tested by tube agglutination with polyclonal anti-Lewis^a and anti-Lewis^b antisera (Dade). All blood specimens were processed within 24 h of collection.

Saliva specimens were frozen within 3 h of collection and transported to Edinburgh for determination of secretor status by the haemagglutination inhibition method (Mollison, 1979) with monoclonal anti-A and anti-B antibodies and *Ulex europaeus* lectin (Sigma) for detection of H antigen (the antigen of blood

group O). Isolation and identification of the *Neisseria* species from the Stonehouse residents has been reported (Cartwright *et al.* 1987).

The results were analysed by the data processing system described previously (Stuart *et al.* 1987).

The Lewis blood groups of 672 randomly selected donors from the area served by the South Western Region Blood Transfusion Service were determined by Dr I. D. Fraser and colleagues (South Western Region Blood Transfusion Service).

RESULTS

ABO blood groups of Stonehouse residents

The distribution of the ABO blood groups among 4899 Stonehouse residents tested is similar to that reported for England (Mourant *et al.* 1976): 2201 group A (44·9%); 411 group B (8·4%); 2147 group O (43·8%); 140 group AB (2·9%).

Secretor status and Lewis blood groups of Stonehouse residents

Saliva specimens were obtained from 4906 (98%) of Stonehouse residents who participated in the study (Table 1). Both the saliva tests and the Lewis blood groups indicate there is a higher proportion of non-secretors in Stonehouse than the predicted 20–25%. By the saliva test, 3554 (72·4%) were secretors and 1352 (27·6%) were non-secretors (Table 2). Of the 15 Stonehouse residents who had had meningococcal infection we examined saliva specimens from 13; 7 of the 13 (54%) were non-secretors.

Of the 672 blood donors from the South Western Regions, 40 (5·9%) were Lewis negative and 632 were Lewis positive; of the remainder 484 (76·5%) were Lewis^b and 148 (23·4%) were Lewis^a. Among the 598 blood specimens from the Stonehouse study tested, 32 (5·3%) were Lewis negative; 7 (22%) were non-secretors and 25 (78%) were secretors by the saliva test. There was no saliva specimens from the blood donors for comparison with those from the study group; therefore, the Lewis negative individuals of both groups were not included in the analysis.

The difference between the distribution of Lewis groups among Stonehouse residents, 276 Lewis^b (67·3%) and 134 Lewis^a (32·7%), and the population in the South Western Region is highly significant ($\chi^2 = 10{\cdot}2$, D.F. = 1, $P < 0{\cdot}005$). The difference between the distribution of Lewis groups among non-Stonehouse residents in the study (e.g. children attending school in Stonehouse), 112 Lewis^b (72%) and 44 Lewis^a (28%), and the South Western Region was not statistically significant $\chi^2 = 1{\cdot}3$, D.F. = 1, $P > 0{\cdot}05$) (Table 3).

Saliva from Lewis^a individuals (non-secretors) may give false positive secretor results if it is contaminated with small amounts of blood as a result of poor oral hygiene or, conversely, over-vigorous brushing of teeth. There was, however, close agreement between the results of the saliva tests and the Lewis blood groups. Among both residents and non-residents tested 389 were Lewis^b and 371 (95·4%) were secretors. The discrepancy might be due to weak expression of the secretor gene or to dilution of very small saliva specimens. Of the 178 Lewis^a individuals, 165 (92·7%) were non-secretors by the saliva test.

Table 1. *Specimens tested from participants in Stonehouse survey for secretor state and Lewis blood groups by area of residence*

	Population	Participants		Saliva		Lewis* blood specimens	Participants (%)
		No.	(%)	No.	(%)		
Park Estate	1729	1273	(73·6)	1264	(99·3)	119	(9·4)
Verney Fields	1836	1216	(66·2)	1184	(97·4)	103	(8·7)
Little Australia	869	750	(86·3)	738	(98·4)	61	(8·3)
Rosedale	1423	1226	(86·2)	1188	(96·9)	102	(8·6)
Bridgend	778	541	(69·5)	532	(98·3)	25	(4·6)
Total	6635	5006	(75·4)	4906	(98)	410	(8·4)

Secretor status and Lewis blood group distribution by area of residence in Stonehouse

The numbers of specimens tested for secretor status and Lewis group for the five areas of Stonehouse are shown in Table 1. Table 2 compares the secretor status and Lewis blood group distributions by area of residence in Stonehouse. While there is close agreement between the saliva tests and Lewis groups for Rosedale and Bridgend areas, the results suggest that the proportion of non-secretors in Park Estate, Little Australia and Verney Fields is underestimated by the saliva test. Except for Rosedale, the proportion of Lewis[a] individuals was over 30%; Park Estate-33%, Verney Fields-36%, Little Australia-38% and Bridgend-33%. There was no significant difference in the proportion of Lewis[a] individuals.

When secretor status was analysed by age bands, the highest proportion of non-secretors was found in the 0–4 and 5–9 age groups (Table 4).

Distribution of secretors and non-secretors in Stonehouse schools

By the saliva test there were no significant differences in the proportions of secretors and non-secretors attending state or private schools in Stonehouse, although most children attending private schools were not Stonehouse residents. The number of pupils for whom Lewis blood group was determined was small, but the results suggest that the numbers of non-secretors were underestimated, particularly among those at the state schools.

Among the schoolchildren tested for Lewis group the proportion of Lewis[a] individuals among the 122 from state junior and state secondary schools was 37% compared with 28% among 75 pupils from the corresponding private schools; but the difference was not statistically significant.

Carriage of Neisseria *species and secretor status*

Among the 69 Stonehouse residents who were carriers of the outbreak strain we examined saliva specimens from 67; there were 47 secretors (70%) and 20 non-secretors (30%). Among the carriers of the non-outbreak strains, 327 (71%) were secretors and 132 (29%) were non-secretors (Table 5).

Tables 6 and 7 include data for carriers resident in Stonehouse as well as non-residents. There were no significant differences in the proportion of non-secretors among the carriers analysed by serogroup (Table 6). There were no differences in the proportion of non-secretors carrying serotypable strains (31%) compared with those carrying non-serotypable strains (31%) (Table 7).

Table 2. *Secretor state and Lewis blood group distributions by area of residence in Stonehouse*

Area	Saliva samples (No.)	Secretors		Non-secretors		Le^a specimens (No.)	Le^b		Le^a		No.* cases
		No.	(%)	No.	(%)		No.	(%)	No.	(%)	
Park Estate	1264	917	(72·5)	347	(27·5)	119	80	(67)	39	(33)	11
Verney Fields	1836	862	(72·8)	322	(27·2)	103	66	(64)	37	(36)	3
Little Australia	738	556	(75·3)	182	(24·7)	61	38	(62)	23	(38)	0
Rosedale	1188	864	(72·7)	324	(27·2)	102	75	(73·5)	27	(26·5)	1
Bridgend	532	355	(66·7)	177	(33·3)	25	17	(69)	8	(32)	0
Total	4906	3554	(72·4)	1352	(27·6)	410	276	(67·3)	134	(32·7)	15

* The cases in each of the areas are included in the figures for the residents.

Table 3. *Secretor state of Stonehouse residents compared with UK as a whole*

	Secretors		Non-secretors			
	No.	(%)	No.	(%)	χ^2	*P*
Saliva test						
Stonehouse	3554	(72·5)	1352	(27·6)		
England (pooled data,* 10 studies)	5747	(76)	1819	(24)	18·4	< 0·0005
Lewis blood group						
Stonehouse	277	(67·3)	134	(32·7)		
England	835	(77·2)	246	(22·8)	14·7	< 0·0005
South West Region	484	(76·6)	148	(23·4)	10·2	< 0·005

* Mourant, Kopec, & Domaniewska (1976).

Table 4. *Secretor state and number of cases in Stonehouse by age group*

		Secretors		Non-secretors		
Age	Number	No.	(%)	No.	(%)	No. cases
0–4	296	199	(67·2)	97	(32·8)	2
5–9	434	299	(68·9)	135	(31·1)	7
10–14	362	275	(76)	87	(24)	4
15–19	384	276	(71·9)	108	(28·1)	0
20–24	308	278	(73·2)	102	(26·8)	0
25–34	807	568	(70·4)	239	(29·6)	1
35–44	674	487	(72·3)	187	(27·7)	0
45–54	439	325	(74)	114	(26)	1
55–64	507	366	(72·2)	141	(27·8)	0
65+	647	462	(71·4)	185	(28·6)	0
Total	4930	3535	(71·7)	1395	(28·3)	15

Table 5. *Distribution of secretors and non-secretors among carriers of meningococci in Stonehouse*

	Secretor		Non-secretor		
Organism	No.	(%)	No.	(%)	Total
Outbreak strains	47	(70)	20	(30)	67
Non-outbreak strains	327	(71)	132	(29)	459

Carriage of *Neisseria lactamica* among Stonehouse residents was analysed with regard to age and secretor status (Table 8). The proportion of non-secretors among the carriers was similar to the proportion of non-secretors in the age band.

DISCUSSION

There is a significantly higher proportion of individuals with Lewis^a blood group in Stonehouse compared with blood donor controls from the South Western Region and other English populations studied (Table 3). Although the saliva test underestimates the numbers of non-secretors, comparison of the results with data

Table 6. *Distribution of secretors and non-secretors among carriers of serogroupable and non-serogroupable* N. meningitidis

Serogroup	Total	Secretors No.	(%)	Non-secretors No.	(%)
Serogroupable		262	(72)	104	(28)
A	3	3		—	
B	211	151	(72)	60	(28)
C	32	23	(72)	9	(28)
W135	22	15	(68)	7	(32)
X	4	4		0	
Y	53	35	(66)	18	(34)
Z	29	22	(76)	7	(24)
Z/29E	19	13	(68)	6	(32)
29E	12	9	(75)	3	(25)
Non-serogroupable	309	209	(68)	100	(32)

Table 7. *Distribution of secretors and non-secretors among carriers analysed by serotype of* N. meningitidis

	Total	Secretors No.	(%)	Non-secretors No.	(%)
Serotypable	288	199	(69)	89	(31)
Serotypes				—	
2a		4			
2b		6		1	
2c		1		—	
P1.16	25	13	(52)	12	(48)
P1.2	77	52	(68)	25	(32)
P1.3	33	27	(82)	6	(18)
15	67	41	(61)	26	(39)
15 P1.16	74	55	(74)	19	(26)
Non-typable	260	180	(69)	80	(31)

from 10 pooled studies of English populations in which saliva was tested for ABH secretion (Mourant *et al.* 1976) shows a higher proportion of non-secretors in Stonehouse.

The relative risk of non-secretors developing meningococcal disease in Stonehouse was 2·4 (95% confidence interval 1·3 to 4·3). This is similar to the relative risk calculated from previous studies in Iceland (1·7), Nigeria (2·9) and Scotland (6·2) (Blackwell *et al.* 1988). If there had been 23% (the South Western Region percentage) instead of 33% non-secretors in Stonehouse with the same attack rate and the same relative risk (2·4), the number of cases would have been 13·6 instead of the 15 observed.

Non-secretors appeared to be more likely to be cases within Park Estate than secretors. In Park Estate 33% of the residents tested were Lewis^a compared with 5 of the 8 cases (62·5%) tested. In Verney Fields 36% of the residents were Lewis^a compared with 2 of the 5 cases (40%). These figures indicate a trend, but are too small for statistical analysis.

Table 8. *Secretor state and age of* N. lactamica *carriers in Stonehouse*

Age	No. isolates	Secretors No.	Secretors (%)	Non-secretors No.	Non-secretors (%)	Non-secretors in Stonehouse (%)
0–4	46	26	(62)	16	(38)	32·8
5–9	20	14	(70)	6	(30)	31·1
10–14	11	6	(55)	5	(45)	24
15–19	11	7	(64)	4	(36)	28·1
20–24	10	7	(70)	3	(30)	26·8
25–34	27	19	(73)	7	(27)	29·6
35–44	7	7		0		27·7
45–54	6	6		0		26
55–64	5	4		1		27·8
65+	4	2		2		28·6
Total	147	98	(69)	44	(31)	

These results suggest that while the proportion of non-secretors who are more susceptible to bacterial meningitis is significantly higher in Stonehouse, it is not sufficient of itself to explain the outbreak.

Our suggestion that blood contamination might account for differences between the saliva tests and Lewis blood groups is supported by detection of Lewis^a antigen in the saliva of a number of 'secretors' by a recently developed ELISA with monoclonal antibodies (unpublished observations). The higher proportion of non-secretors in the under-10 age group might reflect less periodontal disease. In developed countries, periodontal disease is uncommon before puberty, but the frequency of disease begins to rise in the circumpubertal period (11–14 years) (Murray, 1983).

Blood contamination of the saliva could be due to inadequate oral hygiene which is often associated with poor economic background (Waerbaug, 1971: Murray, 1983). There were 21 pairs of blood and saliva specimens from Stonehouse residents in which there was disagreement between the results of the saliva test and the Lewis group. Of the 8 Lewis^a/secretor pairs (false secretors), 7 were from residents of Verney Fields and Park Estate where 14 of the 15 cases of meningitis occurred. The other was from a resident of Little Australia. Of the 13 Lewis^b/non-secretor specimens, 4 were from residents of Park Estate and Verney Fields and 9 from the other three areas.

Agreement between the saliva test and Lewis blood groups was better in areas where socioeconomic levels were higher – Rosedale and Bridgend (Table 3). In Park Estate and Verney Fields where most of the cases of meningococcal disease occurred, the proportion of non-secretors found by the saliva test is lower than that indicated by the proportion of Lewis^a individuals, and there was a higher proportion of household heads in semi-skilled/unskilled occupations. There were no cases of meningococcal disease in Little Australia which had the highest proportion of Lewis^a individuals (38%) but the lowest proportion of non-secretors by the saliva test (24·7%). In this area there was a higher proportion of household heads with skilled jobs than in Park Estate and Verney Fields. The sampling in Little Australia was not as random as in other areas of the town; over half (13/23) of the Lewis^a specimens were from residents in 3 of the 12 postcode areas.

Of the 61 blood specimens tested from Little Australia for Lewis group, there were only 3 in which the saliva test did not agree with the Lewis group: 1 was Lewis^a/secretor and 2 Lewis^b/non-secretor. The number of individuals in Little Australia tested for Lewis group was smaller, which might account for the differences observed for the two tests.

Factors associated with poorer socioeconomic background contribute to susceptibility to infectious diseases. Smoking which might be important in the transfer of meningococci from one individual to another is more prevalent among manual and unskilled workers (Wald *et al.* 1988).

The increased susceptibility of non-secretors to meningococcal infection is not associated with an increased carriage rate of the B15 P1.16 strain. As previous studies (Blackwell *et al.* 1988) have shown a higher proportion of non-secretors among patients with infection due to the three major serogroups of meningococci (serogroups B and C were predominantly isolated from the Icelandic and Scottish patients, serogroups other than B from the Nigerian patients), we did not expect to find an association between secretor status and carriage of any particular serogroups (Table 6). If the protein serotype antigens participate in lectin mediated attachment to carbohydrates on epithelial cells, the carbohydrate moieties of ABO or Lewis^b antigens present in the body fluids of secretors might bind to the bacteria reducing attachment of meningococci to epithelial cells. No consistent patterns was observed; the proportion of non-secretors was the same among carriers of either serotypable or non-serotypable strains.

The prolonged outbreak of disease due to the B15 P1.16 meningococcus in Stonehouse can be partly explained by the significantly higher proportion of non-secretors compared with the South Western Region in general. Our results do not parallel the model suggested for rheumatic fever in which a higher proportion of non-secretors was found among the carriers of group A streptococci (Haverkorn & Gosling, 1969). The increased susceptibility of non-secretors has yet to be explained but it does not appear to be due to increased carriage of these organisms.

We are grateful to Dr I. D. Fraser and his colleagues at the South Western Regional Blood Transfusion Service for determination of the Lewis groups of blood donors from the South West Region and to the Edinburgh and South East Scotland Blood Transfusion Services for reagents used in the study. We are grateful to Mrs M. K. Cole for secretarial assistance in preparation of the manuscript and to Dr J. A. Sofaer, Department of Oral Medicine and Oral Pathology, University of Edinburgh for information on the epidemiology of oral diseases.

This work was supported by grants from the Meningitis Trust and the Department of Health and Social Security.

REFERENCES

BLACKWELL, C. C., JÓNSDÓTTIR, K., HANSON, M., TODD, W. T. A., CHAUDHURI, A. K. R., MATTHEW, B., BRETTLE, R. P. & WEIR, D. M. (1986). Non-secretion of ABO antigens predisposing to infection by *Neisseria meningitidis* and *Streptococcus pneumoniae*. *Lancet* ii, 284–285.

BLACKWELL, C. C., JÓNSDÓTTIR, K., HANSON, M. F. & WEIR, D. M. (1986). Non-secretion of ABO blood group antigens predisposing to infection by *Haemophilus influenzae*. *Lancet* ii, 687.

BLACKWELL, C. C., JÓNSDÓTTIR, K., MOHAMMED, I & WEIR, D. M. (1988). Non-secretion of blood group antigens – a genetic factor predisposing to infection by *Neisseria meningitidis*. In *Gonococci and Meningococci* (eds. J. T. Poolman *et al.*), pp. 633–636. Dordrecht: Martinus Nijhoff.

CARTWRIGHT, K. A. V., STUART, J. M., JONES, D. M. & NOAH, N. D. (1987). The Stonehouse survey, nasopharyngeal carriage of meningococci and *Neisseria lactamica*. *Epidemiology and Infection* **99**, 591–601.

ERIKSSON, A. W., PARTANEN, K., FRANTS, R. R., PRONK, J. C. & KOSTENSE, P. J. (1986). ABH secretion polymorphism in Icelanders, Åland islanders, Finns, Finnish Lapps, Komi and Greenland Eskimos – a review and new data. *Annals of Human Biology* **13**, 273–285.

HANEBERG, B., TANJUN, T., RODAHL, K. & GEDDE-DAHL, J. (1983). Factors preceding the onset of meningococcal disease with special emphasis on passive smoking, stressful events, physical fitness and general symptoms of ill health. *National Institute of Public Health Annals* **6**, 169–174.

HAVERKORN, M. J. & GOSLING, W. R. O. (1969). Streptococci, ABO blood groups and secretor status. *American Journal of Human Genetics* **21**, 360–375.

KAISER, A. B., HENNEKENS, C. H., SASLAW, M. S., HAYES, P. S. & BENNETT, J. V. (1974). Sero-epidemiology and chemoprophylaxis of disease due to sulphonamide-resistant *Neisseria meningitidis* in a civilian population. *Journal of Infectious Diseases* **130**, 217–224.

MOLLISON, P. L. (1979). *Blood Transfusion in Clinical Medicine*, 6th ed., pp. 414–482. Oxford: Blackwell Scientific Publications.

MOURANT, A. E., KOPEC, A. C. & DOMANIEWSKA-SOBSZAK, K. (1976). *The Distribution of the Human Blood Groups and Other Polymorphisms*, 2nd ed., pp. 144–244. London: Oxford University Press.

MURRAY, J. J. (1983). *The Prevention of Dental Disease*, pp. 316–317. Oxford: Oxford University Press.

RACE, R. R. & SANGER, R. (1975). *Blood Groups in Man*, 6th ed., pp. 311–349. Oxford: Blackwell Scientific Publications.

STUART, J. M., CARTWRIGHT, K. A. V., DAWSON, J. A., RICKARD, J. & NOAH, N. D. (1988). Risk factors from meningococcal disease in a case control study in Southwest England. *Community Medicine* **10**, 139–146.

STUART, J. M., CARTWRIGHT, K. A. V., JONES, D. M., NOAH, N. D., WALL, R. J., BLACKWELL, C. C., JEPHCOTT, A. E. & FERGUSON, I. R. (1987). An outbreak of meningococcal disease in Stonehouse: planning and execution of a large-scale survey. *Epidemiology and Infection* **99**, 579–589.

THORDARSON, G., BJARNASON, C., LINCOLN, P. J. & DODD, B. E. (1972). ABH secretor and Lewis type frequencies in an Icelandic series. *Journal of Medical Genetics* **9**, 46–47.

WAERBAUG, J. (1976). Epidemiology of periodontal disease. In *The Prevention of Periodontal Disease*, (ed. J. E. Eastal, D. C. A. Picton and A. G. Alexander), pp. 1–19. London: Henry Kimpton.

WALD, N., KIRYLUK, S., DARBY, S., DOLL, R., PIKE, M. & PETO, R. (1988). *UK Smoking Statistics*, pp. 57–72. Oxford: Oxford University Press.

FEMS Microbiology Immunology 47 (1989) 341-350
Published by Elsevier

FIM 00057

The role of ABO blood groups and secretor status in host defences

C.C. Blackwell

Department of Bacteriology, The Medical School, University of Edinburgh, Edinburgh, U.K.

Received 1 September 1988
Accepted 12 January 1989

Key words: ABO blood groups; Secretor status; Infectious disease; Auto-immune disease

1. SUMMARY

Epidemiological studies on the associations between ABO blood group antigens, secretor status and susceptibility to infectious agents are summarized. Evidence for association of non-secretion with some autoimmune diseases for which infectious aetiologies have been proposed is also given. Several hypotheses are proposed to explain the host-parasite interactions underlying the epidemiological observations, and evidence to support or refute them is presented.

2. INTRODUCTION

'Much more attention should be given to the combined effects of blood group and secretor state on susceptibility to bacterial infections' [1]. The following papers represent current thinking on this previously neglected topic, illustrating the accumulating evidence of its real significance.

In the first article, Dr. Arthur Mourant outlines the epidemiological evidence for associations between ABO blood groups and secretor status with susceptibility not only to infectious diseases but also to other conditions such as carcinoma and ulcers [2]. There is now a relatively large body of evidence for the association of these characters with infectious diseases (Tables 1 and 2) and recently we have found higher proportions of non-secretors among patients with autoimmune conditions such as ankylosing spondylitis, insulin dependent diabetes mellitus and Graves' disease for which infectious aetiologies have been suggested (Table 3).

Among patients with autoimmune diseases for which infectious aetiologies have been postulated, there has been no striking association with ABO groups, but non-secretors are found in significantly higher proportions in a number of these patient categories (Table 3). Particular HLA markers or combinations of markers have been found to be over represented in several of these groups, but the existence of additional genetic susceptibility factors outside the HLA cluster on chromosome 6 has been predicted. The association with non-secretion is the first, albeit indirect, evidence for involvement of infectious agents. Non-secretion might predispose individuals of the susceptible HLA type to the infectious agents that trigger the disease process.

A review of the data on secretor status of different ethnic groups in the context of epidemiological data for some of these autoimmune diseases presents some striking findings. Insulin dependent diabetes is rare among American Indians and Eskimos and compared with Caucasians,

Table 1

Associations between ABO blood groups and susceptibility to infection

Infectious agent/ disease	Blood group association	Reference
Urinary tract		
Escherichia coli	B	51
	B/AB	20
Gastrointestinal tract		
E. coli	B	52
Salmonella and E. coli	B and AB	53
Vibrio cholerae	O	54, 55
Genitourinary tract		
Neisseria gonorrhoeae	B	3
		4
		5
	No association	6
Chlamydia trachomatis	B	Blackwell (unpublished results)
Respiratory tract		
Streptococcus pyogenes Group A	not O	34
Streptococcus pneumoniae	not B	9
Influenza A_2	O	56
Influenza A	O	57
	B	58
Mycobacterium tuberculosis	O	59
Oral cavity		
Periodontal disease	O and AB	60
Candida albicans (carriage)	O	38
Blood borne infection		
Malaria	A	61
Coccidioides immitis	B	26

non-secretors are very much the minority (0–2%) in these populations. The Haida Indians of British Columbia have an unusually high incidence of ankylosing spondylitis, and they are exceptional in that the proportion of non-secretors among them is similar to that of Caucasians (25%) [3]. These observations parallel those of Eriksson et al. [4] that autoimmune diseases and autoantibodies are rare among Lapps and in comparison with non-

Table 2

Associations between non-secretion and susceptibility to infection

Infectious agent	Increased proportion of non-secretors	Reference
Urinary tract		
E. coli	Yes	23
		45
Gastrointestinal tract		
V. cholerae	Yes	62
Genital tract		
C. albicans	Yes	19
C. trachomatis	Yes	Blackwell (unpublished observations)
N. gonorrhoeae	No	8, 9
Respiratory tract		
Str. pyogenes (carriage and rheumatic fever)	Yes	37
Neisseria meningitidis	Yes	16
Str. pneumoniae	Yes	16
Haemophilus influenza	Yes	17
Oral cavity		
C. albicans infections	Yes	19
C. albicans carriage	Yes	38
Periodontal disease	No	60
Caries	Yes	64, 65

Table 3

Association between secretor status and susceptibility to autoimmune diseases

Disease	Increased proportion of non-secretors	Reference
Rheumatic diseases		
Rheumatic fever	Yes	37
Ankylosing spondylitis	Yes	66
Rheumatoid arthritis	No	67
Psoriasis	No	67
Psoriatic arthropathy	Yes	67
Endocrine diseases		
Diabetes mellitus (unclassified)	Yes	68
Insulin dependent diabetes	Yes	49
Non-insulin dependent	No	49
Hashimoto's thyroiditis	No	69
Primary atrophic hypothyroidism	No	69
Graves' disease	Yes	69

Table 4

Distribution of blood-group activity among Gram-negative bacteria [7,11]

Genus	Strains tested	A	B	H(O)	A BH(O)	A B	AH(O)	BH(O)	Inactive
Escherichia	135	8	18	22	6	3	3	4	71
Salmonella	19	1	2	9	0	0	1	0	6
Arizona	3	0	1	1	0	0	0	1	0
Klebsiella	42	2	6	4	3	1	1	5	20
Citrobacter	24	2	2	2	0	2	2	3	11
Pasteurella	8	0	1	0	0	0	0	2	5
Proteus	20	0	6	2	0	0	0	1	11
Pseudomonas	15	1	1	2	0	0	1	0	10
Serratia	2	0	0	2	0	0	0	0	0
Alcaligenes	8	0	0	0	0	0	1	1	6
Shigella	5	0	0	0	0	0	0	0	5
Herrela	1	0	0	0	0	0	0	0	1
N. gonorrhoea	6	6	6	–	–	6	–	–	0

Lapp populations in the same geographic regions, they have lower proportions of non-secretors (2–5%) [5].

Although both blood group and secretor status are stable, easily determined host characters, the host-parasite interactions underlying these epidemiological observations have not previously been actively pursued, probably for several reasons. First, there have been conflicting reports by different groups of researchers. Different blood group associations have been found for the same infection. One explanation is that different strains of microorganisms were present in the populations examined in each study. Foster and Labrum [6] found a higher incidence of the blood group B gene among women attending an antenatal clinic. These were predominantly American black women. A similar increase in the incidence of blood group B individuals among patients with gonorrhoea was reported for British caucasians, but not British blacks [7]. Although there is a higher proportion of individuals of blood group B in the Scottish population we examined, there was a significant increase in the proportion of B individuals among patients with gonorrhoea [8]. In contrast, no unusual distribution of any of the ABO groups was found among male patients with symptomatic gonococcal infections [9]. Different associations with ABO blood groups reported for susceptibility to natural or experimental viral infections might depend on the particular strain examined in the study (Table 1).

The main reason why these studies did not progress much beyond the descriptive epidemiology stage is that there was only one hypothesis suggested to explain the observations. A number of microorganisms have been shown to possess surface antigens that cross react with the A, B and H antigens [7,10,11] (Table 4), and some have been shown to incorporate blood group antigens into their cell envelopes [12]. It was suggested that the anti-A and anti-B isohaemagglutinins might act as 'natural antibodies' for strains with these surface antigens.

Advances in research on protein-carbohydrate interactions involved in pathogenesis of infectious diseases have suggested new approaches for examining the epidemiological findings. Microbial adhesins can bind to peptides, but it appears that carbohydrates are preferentially selected, probably due to the abundance of carbohydrate containing compounds on animal cell surfaces [13]. Since the ABO and Lewis antigens (the expression of the latter is regulated by the secretor gene) are some of the most extensively characterized carbohydrates in man, they are prime candidates for these investigations. A brief review of the genetics, structure and biosynthesis of the blood group

antigens is presented in this volume [14] as well as a review of carbohydrate-protein interactions in pathogenesis of infections [15].

Our recent studies on secretor status and susceptibility to a number of infectious agents suggest that the ability to secrete might be one of the host factors contributing to innate defences, regardless of ABO blood group. The protective effect of the secretor gene is observed particularly among groups of individuals who are immunocompromised in some way. The clearest example is bacterial meningitis. There is a significantly higher proportion of non-secretors among patients with invasive disease due to *Neisseria meningitidis*, *Haemophilus influenzae* and *Streptococcus pneumoniae*, the three pathogens responsible for 75% of bacterial meningitis [16,17]. These are infections that occur predominantly during the period in which maternal antibodies have waned and the child has not yet developed his own active immunity (about 6 months to 4.5 years of age) [18]. A higher proportion of non-secretors was also observed among patients with infections caused by candida species, but this association was noted among those groups who are thought to be immunocompromised in some way, e.g. pregnant women and patients of both sexes with histories of recurrent oral candidiasis [19].

The hypotheses proposed below are not mutually exclusive; these various host-parasite interactions might often overlap in vivo. Details of evidence obtained to support or refute these models are given in papers in this collection. A brief overview of these hypotheses is provided here.

3. ANTI-A AND ANTI-B ISOHAEMAGGLUTININS ARE 'NATURAL' BACTERICIDAL OR OPSONIC ANTIBODIES FOR SOME STRAINS OF MICROORGANISMS

Evidence for this mechanism comes from the work of Reed et al. [12,19] on host susceptibility to infections caused by *Streptococcus pneumoniae* (see [20] for details) and the demonstration of bactericidal and opsonic activity of anti-B for *Escherichia coli* 086 [21,22]. Although there appears to be a synergistic effect between the B gene and non-secretion on susceptibility to recurrent urinary tract infections [23], we were unable to demonstrate any role for isohaemagglutinins as natural antibodies for the 8 serotypes of *E. coli* most commonly isolated from women with urinary tract infections [24].

Our investigation of the possible role of isohaemagglutinins in the association between B blood group and gonorrhoea found that the 'natural' bactericidal activity of human serum for gonococci was not associated with isohaemagglutinins [25]. The anti-A and anti-B antibodies did, however, enhance binding of the bacteria by human monocytes, and those of group B bound greater numbers of the strain tested than monocytes of the other ABO blood groups [26]. In the context of the hypothesis that gonorrhoea is a disease of the human macrophage [27], the enhanced binding/uptake of gonococci by group B monocytes might contribute to the increased susceptibility of B individuals to this disease [26,28]. The greater numbers of bacteria ingested by their monocytes are able to survive the actions of both humoral and cellular defences and form more of the 'infectious units' of bacteria and cellular debris observed in the electron microscopy studies of pus [27].

A similar set of interactions might explain the increased incidence of group B patients with disseminated coccidioidal infections [29] as the yeast expresses a surface antigen that cross reacts with blood group A antigen [30]. The anti-A isohaemagglutinin of B individuals would act as a 'natural opsonin' for the yeasts expressing the A-like antigen on their surfaces.

4. THE ABO OR LEWIS ANTIGENS ARE RECEPTORS FOR ADHESINS ON SOME STRAINS OF MICROORGANISMS

Although there is no direct evidence that the carbohydrate moieties of these antigens are receptors for bacterial adhesins, the P blood group is a receptor for fimbrial adhesins expressed on strains often isolated from patients with pyelonephritis [31] and the Duffy blood group antigen is a receptor for the malaria parasite, *Plasmodium knowlesi*

[32]. We have observed greater binding of *N. gonorrhoeae* to uroepithelial cells from young women of blood group B compared with cells from those of group A [28]. There is also evidence that the Anton antigen is one of the receptors for *H. influenzae* [33,34], and Lewis ^{a} appears to be one of the receptors for some candida isolates [35,36].

5. THE ABH OR LEWIS ^{b} ANTIGENS IN THE BODY FLUIDS OF SECRETORS CAN BIND TO LECTIN-LIKE ADHESINS ON THE SURFACE OF MICROORGANISMS AND INTERFERE WITH THEIR ATTACHMENT TO TARGET CELLS

This might affect carriage or degree of colonization of the mucosa by potentially pathogenic microorganisms. There is a higher proportion of non-secretors among patients with rheumatic fever and also a higher proportion of non-secretors among carriers of group A *Streptococcus pyogenes* [37]. A significant increase in the proportion of individuals of group O and of non-secretors of all ABO blood groups was found among carriers of *Candida albicans* in a healthy population [38].

One of the properties of lectin adhesins is their inhibition by the specific sugars involved in their protein-carbohydrate interactions. If the ABH or Lewis ^{b} antigens are host receptors for some of the adhesins involved in colonization, the glycoprotein forms of these substances in the body fluids of secretors might inhibit the ability of the microorganism to bind to the host's mucosa. As early as 1975, inhibition of attachment of *Streptococcus salivarius* to epithelial cells by salivary glycoproteins with blood group activity had been reported [39], and we have found heat-treated saliva from secretors to inhibit binding of candida to epithelial cells [19].

6. THE LEWIS ^{a} ANTIGEN FOUND PREDOMINANTLY ON THE CELLS OF NON-SECRETORS IS ONE OF THE RECEPTORS FOR LECTIN-LIKE ADHESINS OF SOME MICROORGANISMS

Lomberg et al. [40] found that binding of uropathogenic strains of *E. coli* to target cells was not associated with the density of the P antigens. They observed that higher numbers of these bacteria bound to cells obtained from non-secretors. Since one of the major antigenic differences between secretors and non-secretors is the predominance of Lewis ^{a} in non-secretors, we examined the hypothesis that blocking the Lewis ^{a} antigen might reduce binding of some strains of microorganisms to non-secretor cells. Our evidence suggests Lewis ^{a} might be one of the receptors for some strains of candida [35,36].

7. A PRODUCT OF THE SECRETOR GENE ALTERS THE RECEPTOR FOR SOME MICROBIAL ADHESINS OR MOLECULES NEAR THESE RECEPTORS REDUCING THE NUMBERS OF MICROORGANISMS BOUND

There is as yet no published evidence for this hypothesis but it was suggested by Lomberg and coworkers to explain the higher numbers of bacteria bound to cells of non-secretors [40].

8. THE LOWER LEVELS OF SERUM [41] AND SALIVARY IgA [42] REPORTED FOR NON-SECRETORS CONTRIBUTES TO A COMPROMISED STATE AT THEIR MUCOSAL SURFACES

The mass screening of the residents of Stonehouse for carriage of meningococci [43] provided an opportunity to examine the levels of serum and salivary antibodies among carriers and non-carriers matched for age, sex and secretor status. We were unable to confirm the report of lower levels of IgA in the saliva of non-secretors for whom no neisseriae were isolated. The levels of IgA in the saliva of both secretor and non-secretor carriers of *N. meningitidis* were however significantly higher than those of non-carriers [44]. The levels of salivary IgA among secretors or non-secretors carrying the non-pathogenic *Neisseria lactamica* did not differ from those of the non-carriers [20].

We were able to confirm the reported lower serum IgA levels among non-secretors from whom no neisseriae were isolated. The non-secretors from

whom serogroupable strains were isolated had significantly increased levels of IgA than the non-secretors who did not carry meningococci [20]. The possible role of serum IgA acting as blocking antibodies is discussed in the article in this volume on bacterial meningitis [20].

In contrast to our predictions that non-secretors might have impaired IgA responses, non-secretor women with recurrent urinary tract infections had significantly higher levels of total serum IgA than the secretors. The levels of total serum IgA among non-secretors who had improved over the 20 year period were higher than those found for secretors who had improved. No differences were found between these immunoglobulin levels for secretors and non-secretors who had not improved or in whom there was no change in the number of infections [45]. The results of both studies suggested that there is a difference in the IgA responses of secretors and non-secretors to colonization/infection and that non-secretors might be more dependent on their specific immune responses than secretors to deal with these infections.

These apparent differences in the specific immune responses of secretors and non-secretors require further investigation for their possible contributions to prevention of infections and also to their possible contributions to pathogenesis of some infections [20,46].

9. AS THE SECRETOR GENE AND THE STRUCTURAL GENE FOR THE THIRD COMPONENT OF COMPLEMENT (C3) ARE IN THE SAME LINKAGE GROUP, THERE MIGHT BE DIFFERENCES IN THE LEVELS OF C3 FOUND IN SECRETORS AND NON-SECRETORS

Among the group from whom no neisseriae were isolated in the Stonehouse study, the levels of C3 found for non-secretors were slightly lower than those of the secretors. In this group there were 8 individuals in whom C3 levels were below the 'normal' range; 7 of these were non-secretors [44]. The donors with these low levels of C3 were not the very young children in the survey (Fig. 1).

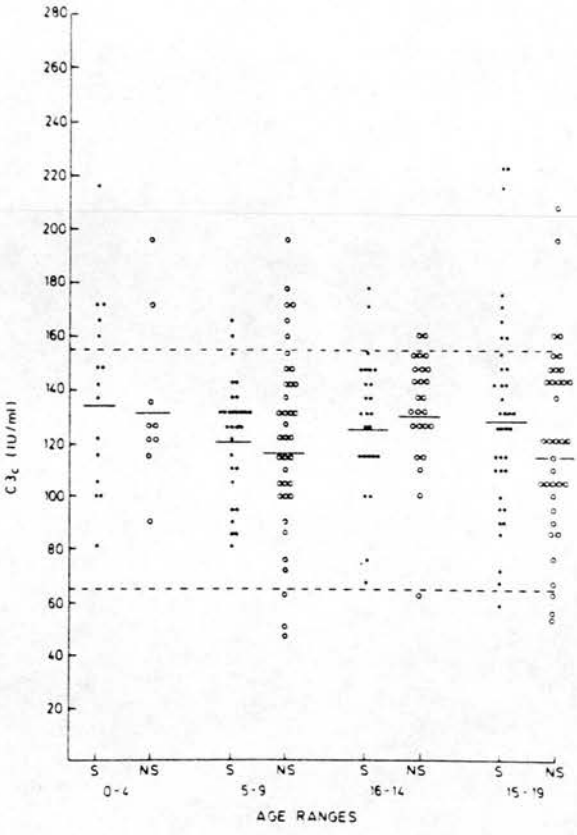


Fig. 1. C3c levels of secretors and non-secretors.

Lower levels of C3 and C4 complement components have been found among patients with insulin dependent diabetes [47] but not among those with non-insulin dependent diabetes [48]. Our finding of a significant increase in the proportion of non-secretors among insulin dependent diabetics [49] prompted us to determine their C3 and C4 levels. Both these complement components were significantly lower in the non-secretors [50]. Any functional differences in the activities of C3 and C4 between secretors and non-secretors in the patient and control groups remain to be determined.

10. CONCLUSIONS

The main value of these studies is that they provide stable host markers that can be used in

investigation of host-parasite interactions involved in a number of infectious diseases as well as intriguing observations such as the one alluded to by Dr. Mourant — the association between the old observation of an increase in the proportion of non-secretors among patient with ulcers and the newer association of ulcers with the presence of *Campylobacter pylori* [1]. Isolation of adhesins on microorganisms that recognize blood group antigens and assessment of their immunogenicity might provide new approaches to prevention of colonization and subsequent infections. Examination of the differences in levels and functional activity of C3 and C4 complement components of secretors and non-secretors will be of value in studies of the susceptibility to infection, particularly among patients with diabetes. The differences between the immune responses of secretors and non-secretors following natural infections or immunization might provide new insights into (1) the role of different isotypes in relation to pathogenesis and prevention of infections, (2) the role of different isotypes in development and clearance of immune complexes in the pathogenic sequelae of infections (3) the role of infectious agents as triggers for some autoimmune diseases.

Future work in this area should include examination of patients who are immunosuppressed to determine if secretion of blood group antigens plays a role in prevention of opportunistic infections. These would include individuals receiving chemotherapy or radiotherapy or those undergoing organ or bone marrow transplants. Of particular interest would be individuals with HIV infections. Because the route of infection in intravenous drug users bypasses the defences of the mucosa, one would predict the proportion of secretors and non-secretors with antibodies to the virus in this group would reflect those of the local population. Non-secretors whose immunity to a specific pathogen is absent or reduced (e.g., pregnancy) appear to be more susceptible to infection. Non-secretors with HIV infections might be those who have problems with opportunistic pathogens earlier in the course of the disease as their immune responses become compromised.

No work has been published on secretor status and viral infections. Such studies might be of considerable value not only in elucidating host factors influencing susceptibility to viral infection but also in examining the role of viruses in the development of invasive bacterial diseases such as pneumonia and meningitis, autoimmune diseases such as insulin dependent diabetes and malignant disorders for which there is an association with non-secretion.

REFERENCES

[1] Mourant, A.E. (1983) Blood relations. Blood groups and anthropology. p. 129, Oxford University Press, London.

[2] Mourant, A.E. (1989) Recent advances in the study of associations between infection and genetic markers. FEMS Microbiol. Immunol. 47, 317–320.

[3] Mourant, A.E., Kopec, A.C. and Domaniewska-Sobczak, K. (1976) The distribution of human blood groups and other polymorphisms. Oxford University Press, London.

[4] Eriksson, A.W., Lehmann, W. and Simpson, N.E. (1979) Genetic studies on circumpolar populations, in The human biology of circumpolar populations (Milan, F.A., ed.), pp. 81–168, Cambridge University Press, U.K.

[5] Eriksson, A.W., Partanen, K., Frants, R.R., Pronk, G.C., Kostense, P.J. (1986) ABH secretion polymorphism in Icelanders, Aland Islanders, Finns, Finnish Lapps, Komi and Greenland Eskimos: a review and new data. Ann. Hum. Biol. 3, 273–285.

[6] Foster, M.T. and Labrum, A.H. (1976) Relation of infection with *Neisseria gonorrhoeae* to ABO blood groups. J. Infect. Dis. 133, 329–330.

[7] Miler, J.J., Novotny, P., Walker, P.D., Harris, J.R.W. and MacLennan, I.P.B. (1977) *Neisseria gonorrhoeae* and ABO isohaemagglutinins. Infect. Immun. 15, 713–719.

[8] Kinane, D.F., Blackwell, C.C., Winstanley, F.P. and Weir, D.M. (1983) Blood group, secretor status and susceptibility to infection by *Neisseria gonorrhoeae*. Br. J. Vener. Dis. 59, 44–46.

[9] Johnson, A.P., Osborn, M.F., Hanna, N.F., Dawson, S.G., McManus, T.J. and Taylor-Robertson, D. (1983) A study of the relationship between ABO blood groups, secretor status and infection with *Neisseria gonorrhoeae*. J. Infect. 6, 171–174.

[10] Springer, G.F. (1970) Importance of blood-group substances in interactions between man and microbes. Ann. N.Y. Acad. Sci. 169, 134–152.

[11] Springer, G.F., Williamson, P. and Brandes, W.C. (1971) Blood group activity of Gram-negative bacteria. J. Exp. Med. 113, 1077–1093.

[12] Reed, W.P., Drach, G.W. and Williams, R.C., Jr. (1974) Antigens common to human and bacterial cells. IV. Studies of human pneumococcal disease. J. Lab. Clin. Med. 83, 599–610.

[13] Lark, D. (1986) Protein-carbohydrate interactions in biological systems. Academic Press, London.

[14] Green, C. (1989) The ABO, Lewis and related blood group antigens: a review of structure and synthesis. FEMS Microbiol. Immunol. 47, 321–330.

[15] Weir, D.M. (1989) Carbohydrates as recognition molecules in infection and immunity. Microbiol. Immunol. 47, 321–330.

[16] Blackwell, C.C., Jonsdottir, K., Hanson, M., Todd, W.T.A., Chaudhuri, A.K.R., Mathew, B., Brettle, R.P. and Weir, D.M. (1986) Non-secretion of ABO antigens predisposing to infection by *Neisseria meningitidis* and *Streptococcus pneumoniae*. Lancet ii, 284–285.

[17] Blackwell, C.C., Jonsdottir, K., Hanson, M.F. and Weir, D.M. (1986) Non-secretion of ABO antigens predisposing to infection by *Haemophilus influenzae*. Lancet ii, 687.

[18] Greenwood, B.M. (1984) Selective primary health care: strategies for control of disease in the developing world XIII Acute bacterial meningitis. Rev. Infect. Dis. 6, 374–389.

[19] Thom, S.M., Blackwell, C.C., MacCallum, C.J., Weir, D.M., Brettle, R.P., Kinane, D.F. and Wray, D. (1989) Nonsecretion of ABO blood group antigens and susceptibility to infection by *Candida* species. FEMS Microbiol. Immunol. 47, 401–406.

[20] Blackwell, C.C., Jonsdottir, K., Weir, D.M., Hanson, M.F., Cartwright, K.A.V., Stuart, J.M., Jones, D.M. and Mohammed, I. (1989) Blood group, secretor status and susceptibility to bacterial meningitis. FEMS Microbiol. Immunol. 47, 351–356.

[21] Check, J.H., O'Neill, E.A., O'Neill, K.E. and Fuscaldo, K.E. (1972) Effect of anti-B antiserum on the phagocytosis of *Escherichia coli*. Infect. Immun. 6, 95–96.

[22] Muschel, L.H. and Osawa, E. (1959) Human blood group substance B in *Escherichia coli* 086. Proc. Soc. Exp. Biol. N.Y. 101, 614–617.

[23] Kinane, D.F., Blackwell, C.C., Brettle, R.P., Weir, D.M., Winstanley, F.P. and Elton, R.A. (1982) ABO blood group, secretor state and susceptibility to recurrent urinary tract infections in women. Br. Med. J. 185, 7–9.

[24] Blackwell, C.C., Andrew, S., May, S.J., Weir, D.M., MacCallum, C.J. and Brettle, R.P. (1984) ABO blood groups and susceptibility to urinary tract infections: no evidence for involvement of isohaemagglutinins. J. Clin. Lab. Immunol. 15, 191–194.

[25] Winstanley, F.P., Blackwell, C.C., Weir, D.M. and Kinane, D.F. (1983) ABO blood groups and susceptibility to gonorrhoea II. The relationship of lipopolysaccharide type to gonococcal sensitivity to the bactericidal activity of normal human serum. J. Clin. Lab. Immunol. 11, 27–32.

[26] Kinane, D.F., Blackwell, C.C., Weir, D.M. and Winstanley, F.P. (1983) ABO blood groups and susceptibility to gonococcal infection III. Role of isohaemagglutinins in increased association of *Neisseria gonorrhoeae* to monocytes from blood group B individuals. J. Clin. Lab. Immunol. 12, 83–86.

[27] Novotny, P., Short, J.A., Hughes, M., Miler, J.J., Syrett, C., Turner, W.H., Harris, J.R.W. and MacLennan, I.P.B. (1977) Studies on the mechanisms of pathogenicity of *Neisseria gonorrhoeae*. J. Med. Microbiol. 10, 346–365.

[28] Blackwell, C.C., Winstanley, F.P., Weir, D.M. and Kinane, D.F. (1985) Host-parasite interactions influencing susceptibility to diseases caused by the pathogenic *Neisseria* species, in The Pathogenic Neisseriae (Schoolnik, G.K., Brooks, G.F., Falkow, S., Frasch, C.E., Knapp, J.S., McCutchen, J.A. and Morse, S.A., eds.), pp. 452–455, ASM Publications, Washington, D.C.

[29] Derenski, S.C., Papagianis, D. and Stevens, D.A. (1979) Association of ABO blood groups and outcome of coccidioidal infection. Sabouraudia 17, 261–264.

[30] Landau, J.W., Fujiwara, A., Newcome, V. and Dzawachiszwili, N. (1964) The relationship of commonly encountered saprophytic and pathogenic fungi to human A and B blood group antigens. J. Invest. Dermatol. 43, 59–60.

[31] Kallenius, G., Mollby, R., Svensson, S.B., Winberg, J., Lundblad, A., Svenson, S. and Cedergren, B. (1980) The pk antigen as receptor for the haemagglutinin of pyelonephritic *Escherichia coli*. FEMS Microbiol. Lett. 7, 297–302.

[32] Miller, L.H., Mason, A.J., Dvorak, J.A., McGinniss, M.H. and Rothman, I.K. (1975) Erythrocyte receptors for (*Plasmodium knowlesi*) malaria: Duffy blood group determinants. Science 189, 561–563.

[33] Van Alphen, L., Poole, J. and Overbeeke, M. (1986) The Anton blood group antigen is the erythrocyte receptor for *Haemophilus influenzae*. FEMS Microbiol. Lett. 37, 69–71.

[34] Van Alphen, L., Van Ham, M., Geelen-Van den Broek, L. and Pieters, T. (1989) Relationship between secretion of the Anton blood group antigen in saliva and adherence of *Haemophilus influenzae* to oropharynx epithelial cells. FEMS Microbiol. Immunol. 47, 357–362.

[35] May, S.J., Blackwell, C.C. and Weir, D.M. (1987) Non-secretion of blood group antigens and susceptibility to *Candida albicans*: the role of Lewis blood group antigens. J. Dent. Res. 65, 503.

[36] May, S.J., Blackwell, C.C. and Weir, D.M. (1989) Lewis ^{a} blood group antigen of non-secretors: a receptor for *Candida* blastospores. FEMS Microbiol. Immunol. 47, 407–410

[37] Haverkorn, M.J. and Goslings, W.R.O. (1969) Streptococci, ABO blood groups and secretor status. Am. J. Hum. Genet. 21, 360–375.

[38] Burford-Mason, A.P., Weber, J.C.P. and Willoughby, J.M.T. (1988) Oral carriage of *Candida albicans*, ABO blood group and secretor status in healthy subjects. J. Med. Vet. Mycol. 26, 49–56.

[39] Williams, R.C. and Gibbons, R.J. (1975) Inhibition of streptococcal attachment to receptors on human buccal epithelial cells by antigenically similar salivary glycoproteins. Infect. Immun. 11, 711–718.

[40] Lomberg, H., Cedergren, B., Leffler, H., Nilsson, B., Carlstrom, A.-S. and Svanborg-Eden, C. (1986) Influence of blood group on the availability of receptors for attachment of uropathogenic *Escherichia coli*. Infect. Immun. 51, 919–926.

[41] Grundbacher, F.J. (1972) Immunoglobulins, secretor status and the incidence of rheumatic heart disease. Hum. Hered. 25, 399–404.

[42] Waissbluth, J.G. and Langman, J.S. (1971) ABO blood group, secretor status, salivary protein, and serum and salivary immunoglobulin concentrations. Gut. 12, 646–649.

[43] Stuart, J.M., Cartwright, K.A.V., Jones, D.M., Noah, N.D., Wall, R.J., Blackwell, C.C., Jephcott, A.E., Ferguson, I.R. (1987) An outbreak of meningococcal disease in Stonehouse: planning and execution of a large scale survey. Epidemiol. Infect. 99, 579–589.

[44] Blackwell, C.C., Weir, D.M., James, V.S., Cartwright, K.A.V., Stuart, J.M. and Jones, D.M. (1988) Non-secretion of ABO blood group antigens and susceptibility to infectious diseases. NATO Advanced Workshop. Bacteria and the phagocytic cell, pp. 357–370.

[45] Blackwell, C.C., May, S.J., Brettle, R.P., MacCallum, C.J. and Weir, D.M. (1987) Secretor state and immunoglobulin levels among women with recurrent urinary tract infections. J. Clin. Lab. Immunol. 22, 133–137.

[46] May, S.J., Blackwell, C.C., Brettle, R.P., MacCallum, C.J. and Weir, D.M. (1989) Non-secretion of ABO blood group antigens: a host factor predisposing to recurrent urinary tract infections and renal scarring. FEMS Microbiol. Immunol. 47, 383–388.

[47] Charlesworth, J.A., Timmermans, V., Golding, J., Campbell, L.V., Peake, P.W., Pussell, B.A., Wakefield, D. and Howard, N. (1987) The complement system in type i (insulin-dependent) diabetes. Diabetologia 30, 372–379.

[48] Charlesworth, J.A., Campbell, L.V., Catanzaro, R., Pussel, B.A., Pasterfield, C.V. and Peake, P.W. (1982) Immune complexes in diabetes mellitus: studies of complement utilization and tissue deposition. J. Clin. Lab. Immunol. 8, 163–168.

[49] Blackwell, C.C., James, V.S., Weir, D.M., Gemmill, J.D., Patrick, A.W., Collier, A. and Clarke, B.F. (1987) Secretor state of patients with insulin-dependent or non-insulin-dependent diabetes mellitus. Br. Med. J. 295, 1024–1025.

[50] Blackwell, C.C., Weir, D.M., Patrick, A.E., Collier, A. and Clarke, B.F. Secretor state and complement levels (C3, C4) in insulin dependent diabetes mellitus. Diabet. Res. (in press).

[51] Cruz-Coke, R., Paredes, L. and Montenegro, A. (1965) Blood groups and urinary microorganisms. J. Med. Genet. 2, 185–188.

[52] Socha, W., Bilinska, M. and Kaczera, A. (1969) *Escherichia coli* and ABO blood groups. Folia Biol. (Krakola) 17, 259–269.

[53] Robinson, M.G., Tolchin, D. and Halpern, C. (1971) Enteric bacteria and the ABO blood groups. Am. J. Hum. Genet. 23, 135–145.

[54] Barua, D. and Paguio, A.S. (1977) ABO blood groups and cholera. Ann. Hum. Biol. 4, 489–492.

[55] Chaudhuri, A. and De, S. (1977) Cholera and blood groups. Lancet ii, 404.

[56] McDonald, J.C. and Zukerman, A.J. (1962) ABO blood groups and acute respiratory virus disease. Br. Med. J. ii, 89–90.

[57] Potter, C.W. (1969) Haemagglutination inhibition antibody to various influenza viruses and adenoviruses in individuals of blood groups A and O. J. Hyg. 67, 67–74.

[58] Mackenzie, J.S. and Fimmel, P.J. (1978) The effect of ABO blood groups on the incidence of epidemic influenza and on the response to live attenuated and detergent split influenza vaccines. J. Hyg. 80, 21–30.

[59] Viskum, K. (1975) The ABO and Rhesus blood groups in patients with pulmonary tuberculosis. Tubercle 56, 329–334.

[60] Pradhan, A.C., Chawla, T.N., Samuel, K.C. and Pradhan, S. (1971) The relationship between periodontal disease and blood groups and secretor status. J. Periodontal Res. 6, 294–300.

[61] Gupta, M. and Chowdhuri, A.N.R. (1980) Relationship between ABO blood groups and malaria. Bull. WHO 58, 913–915.

[62] Chaudhuri, A. and Das Adhikary, C.R. (1978) Possible role of blood group secretory substances in the aetiology of cholera. Trans. Roy. Soc. Trop. Med. Hyg. 72, 664–665.

[63] Blackwell, C.C., Thom, S.M., Lawrie, O., Weir, D.M., Wray, D. and Kinane, D.F. (1986) Non-secretion of blood group antigens and susceptibility to oral infections due to *Candida albicans*. J. Dent. Res. 65, 502.

[64] Arneberg, P., Kornstad, L., Nordbo, H. and Gjermo, P. (1976) Less dental caries among secretors than among nonsecretors of blood group substance. Scand. J. Dent. Res. 84, 362–366.

[65] Holbrook, W.P. and Blackwell, C.C. (1989) Secretor state and dental caries in Iceland. FEMS Microbiol. Immunol. 47, 397–400.

[66] Shinebaum, R., Blackwell, C.C., Forster, P.J.G., Hurst, N.P., Weir, D.M. and Nuki, G. (1987) Non-secretion of ABO blood group antigens as a host susceptibility factor in the spondylarthropathies. Br. Med. J. 294, 208–210.

[67] Shinebaum, R., Blackwell, C.C., Abernethy, V.E., Situnayake, D.R., Weir, D.M. and Nuki, G. (1987) Secretor state of patients with psoriasis, psoriatic arthropathy or rheumatoid arthritis. Br. J. Rheumatol. 76 (suppl. 2), 105.

[68] Andersen, J. and Lauritzen, E. (1960) Blood groups and diabetes. Diabetes 9, 20–24.

[69] Collier, A., Patrick, A.W., Toft, A.D., Blackwell, C.C., James, V.S. and Weir, D.M. (1988) Increased prevalence of non-secretors in patients with Graves' disease: evidence for an infective aetiology? Br. Med. J. 296, 1162.

FEMS Microbiology Immunology 47 (1989) 315-316
Published by Elsevier

FIM 00053

A.E. Mourant: a biographical note in celebration of his 85th birthday

Arthur Ernest Mourant was born in Jersey 11 April 1904 and was educated there at Victoria College. In 1922 he went up to Exeter College, Oxford with a King Charles I scholarship where he obtained a first class honours degree in Chemistry in 1926. He was awarded the Burdett-Coutts Scholarship for geological research and received his D. Phil in Geology from Oxford in 1931.

For two years he was a probationer geologist with the Geological Survey of Great Britain. Fortunately for the field of blood groups and serology, he failed to be awarded a Commonwealth Fellowship for a research project to seek evidence in the Eastern United States for the theory of continental drift!

At the age of 34 he became a medical student at St. Bartholomew's Hospital and received his MBChB degree from Oxford in 1943 followed by his M.D. in 1948. The problems generated by the increased demand for blood transfusion during World War II largely involved the new field of serology. Arthur Mourant was a pioneer in this area as a medical officer in the National Blood Transfusion Service, during which time he discovered two new blood groups. There followed a period of research at Cambridge with R.R. Race and R.R.A. Coombs, when the Coombs antiglobulin test was developed and should perhaps be more correctly known as the Coombs-Mourant-Race test, as all three were involved in the work. This test was central to the methods used for the discovery by Race, Mourant and others of many of the blood groups now known.

In 1946, Arthur Mourant became the founder director of the Blood Group Reference Laboratory, later recognised by the World Health Organization as the International Blood Group Reference Laboratory. His interest in the distribution of the blood group polymorphisms continued and he compiled the published and unpublished studies in several books that have been of compelling interest to physical anthropologists as well as to biomedical scientists and clinicians. One remarkable fact is that the following texts were all prepared before the availability of computerized databases: The Distribution of Human Blood Groups (1954); The ABO Blood Groups, Comprehensive Tables and Maps of World distribution (1958); The Distribution of the Human Blood Groups and Other Biochemical Polymorphisms (1976); Blood Groups and Diseases (1978) and The Genetics of the Jews (1978).

The honours he has received reflect the wide range of his contributions: Oliver Memorial Award for services to Blood Transfusion (1952); President, Section H (Anthropology), British Association for the Advancement of Science (1956); Huxley Memorial Medal, Royal Anthropological Institute, London (1961); Fellow of the Royal Society of London (1966); Landsteiner Memorial Award, American Association of Blood Banks (1973); Marett Memorial Lecture, Exeter College, Oxford (1978); Worth Award, Geological Society of London (1982); Osler Memorial Medal, University of Oxford (1980); Honorary Citizen of Toulouse (1985). He is an honorary member of Sociedad Peruana de Patologia, Société Jersiaise, International Society of Blood Transfusion, British Society for Haematology, Society for the Study of Human Biology, and the first and only honorary member of the Human Biology Council. He has been or still is a member of the editorial board of 7 scientific journals.

In 1976 Arthur Mourant officially retired as Director of the Serological Population Genetics Laboratory in London, but he has continued to follow his various interests from his 'retirement' home in Jersey. In 1982 I joined the worldwide network of his correspondents and have often wondered how he finds time for retirement activities such as photography and the local historical, archaeological and geological societies.

I, and I am sure many others, have benefited from his advice and encyclopaedic memory for early references. This special issue is an attempt to bring together the recent advances on the association of blood groups and secretor status with infectious diseases which he suggested many years ago would be a fruitful area of investigation. With its publication go all good wishes for a happy 85th birthday and the continued success of a very remarkable 'retirement'.

C.C. Blackwell
24 August 1988

FEMS Microbiology Immunology 47 (1989) 383–388
Published by Elsevier

FIM 00063

Non-secretion of ABO blood group antigens: a host factor predisposing to recurrent urinary tract infections and renal scarring

S.J. May, C.C. Blackwell, R.P. Brettle [1], C.J. MacCallum and D.M. Weir

Department of Bacteriology, The Medical School, University of Edinburgh, and [1] Infectious Diseases Unit, City Hospital, Edinburgh, Scotland, U.K.

Received 1 September 1988
Accepted 12 January 1989

Key words: Blood group antigens; ABO antigens; Urinary tract infection; Renal scars

1. SUMMARY

In a study of 718 women referred for specialist investigation for recurrent urinary tract infections, 250 (34.8%, $P < 0.01$) were non-secretors. The proportion of non-secretors among the women with renal scars (42.6%) was higher than that found for women with no evidence of renal scars (36.6%). Among 29 patients in whom symptoms began in childhood or adolescence, 51.7% were non-secretors. The proportion of non-secretors among individuals with renal scars in this study (42.6%) and that reported in the accompanying paper for Swedish children (40%) suggests that non-secretion might influence the pathogenic sequelae of these infections. Possible host–parasite interactions underlying the increased proportion of non-secretors among women with recurrent urinary tract infections and those leading to development of renal scars are discussed.

Correspondence to: C.C. Blackwell, Department of Bacteriology, The Medical School, University of Edinburgh, Edinburgh, U.K.

2. INTRODUCTION

Recurrent urinary tract infections are common problems among women and girls. It has been estimated that approximately 50% of women will have at least one urinary tract infection during their lives [1]. Despite the availability of antibacterial agents with good in vitro activity, recurrent infections occur in a number of women. Some failure in the host defences has been suggested to underly this problem [2].

Although uncomplicated lower urinary tract infections rarely lead to renal damage [1], infections of the upper urinary tract can result in renal scarring. Scarring following urinary tract infections occurs in young children, most of whom are under the age of 5 [3]. This is the age range in which maternal antibody has waned and the child's immune system is not fully developed. It is also the same age range in which bacterial meningitis is most prevalent [4]. The increased proportion of non-secretors among patients with bacterial meningitis suggested secretion of blood group antigens might play a role in the host's innate defences against infection during this vulnerable

Table 1

Secretor state of patients with urinary tract infections

	Reference	Total no.	No. non-secretor	% non-secretor	*P*
Adults					
Edinburgh study	[6]	718	250	34.8	< 0.01
20 year study group		202	78	38.6	< 0.01
without renal scars		146	53	36.6	< 0.05
with renal scars		54	23	42.6	< 0.01
juvenile onset		29	15	51.7	< 0.01
controls	[8]	334	89	26.6	
Children					
Swedish study	[7]				
patients with UTI		77	23	29	NS
without renal scars		42	9	21	NS
with renal scars		35	14	40	< 0.05
controls	[27]	954	208	21.3	

NS, not significant.

period. There was, indeed, a significantly higher proportion of non-secretors among the women in our study with juvenile onset of symptoms (51.7%) and among those with renal scars (42.6%) [5,6].

The proportion of non-secretors among our patients with renal scars is similar to those reported in the accompanying study of 77 Swedish children (40%) [7] (Table 1). Here we wish to consider possible host–parasite interactions responsible for the increased susceptibility of non-secretors to recurrent infections and those that might result in development of renal scars following urinary tract infection.

3. EPIDEMIOLOGY

3.1. Host factors

In comparison with local controls (26.6%) [8], there was a significant increase in the proportion of non-secretors (34.8%, $P < 0.01$) among women referred to the Pyelonephritis Clinic for investigation of recurrent urinary tract infections. Among Swedish children followed from their first known episode of pyelonephritis, there was an increase in the proportion of non-secretors (29%), but this increase was not statistically significant [7]. Determination of secretor status of a larger number of children with urinary tract infections might provide results similar to those for adults.

Among patients in both groups with renal scars, the proportion of non-secretors was significantly increased, 42.6% for the Scottish women and 40% for the Swedish girls. As renal scarring usually occurs before the age of 5, scarring found among adults probably reflects damage during childhood. None of the women in the study developed scars during the 20 years they were followed [9].

The results of these two studies support the concept that there are common host factors in the subset of individuals who develop scars. Host factors such as vesicoureteral reflux [10] and age [3] have been identified. Both indicate that non-secretion is another factor found among a substantial proportion of individuals who sustain this type of kidney damage.

3.2. The bacteria

In both the Scottish [6,11] and Swedish studies [7], the numbers of infectious episodes among the patients with scars did not differ from those without scars. Although we had predicted the more virulent strains would be isolated from the more susceptible non-secretors, our study characterizing *E. coli* strains from secretors or non-secretors found that fewer of the strains from non-secretors expressed mannose-resistant haemagglutinins and/or haemolysin. Similar observations have been made for isolates from the Swedish children. The strains obtained from children who developed

renal scars were not those with the virulence associated mannose-resistant fimbriae that attach to the P blood group antigen. [Lomberg et al., manuscript in preparation]. These studies have led both our group and the Göteborg group to consider that the host's response to the invading strain might play a significant role in the pathogenesis of kidney scarring.

3.3. The immune responses of secretors and non-secretors to urinary pathogens

It has been suggested that the ability to secrete the ABO antigens might play a role in the host's innate defences influencing attachment/colonization of the urinary tract mucosa by pathogenic bacteria [11–13]. Evidence is accumulating to suggest that secretor status might also influence both the inflammatory responses and the specific immune response to uropathogenic strains of *E. coli* [6].

The C3 gene is located in the same linkage group as the secretor gene. We found lower levels of C3 among non-secretors, but the differences were not significant. Among the 8 individuals in whom C3 was below the normal range, 7 were non-secretors [13,14]. As C3 plays a role in defending the host from infection by both the classical and the alternative complement pathways, this might be a useful area to explore.

Lower levels of both serum [15] and secretory [16] IgA have been reported for non-secretors. We tested the hypothesis that if non-secretors responded less effectively to colonization/infection of their mucosal surfaces, this might contribute to their susceptibility to recurrent urinary tract infections. Secretory IgA levels among young girls with recurrent urinary tract infections were reported to be lower than those of age matched controls [17].

The results of the study refuted this hypothesis. Among the women followed for 20 years by the local pyelonephritis clinic, non-secretors had higher levels of both IgA and IgG. This suggested that they were more dependent than secretors on their specific immune responses. Regardless of the age at which symptoms of infection began or the therapy prescribed for these patients, the majority (127/202) improved over the 20 years. They had fewer episodes of infection during the second decade compared with the number during the first decade. Among those who improved, non-secretors had significantly higher levels of IgA and IgG compared with those found for secretors. Among those who had not improved, there was no difference in levels of IgA and IgG of secretors and non-secretors [6].

A review of the literature found that serum antibodies specific for some pathogens appear to influence their carriage. Reduction in carriage of serogroup C meningococci, but not other serogroups, followed immunization of recruits with group C polysaccharide [18]. The probability of becoming a carrier of serogroup B meningococci was greater in recruits who lacked antibodies to serogroup B at induction [19]. Candida was isolated more frequently from women with low levels of anti-candida IgA. The levels of anti-candida IgG were not associated with isolation of the yeast [20]. A significant correlation between anti-candida IgA antibodies in serum and secretions has been reported [21]. Investigation of the *E. coli* antigens to which the increased levels of IgA in non-secretors are directed might identify those that induce protection from reinfection.

Two hypotheses arise from these observations. First, in the non-immune secretor, glycoproteins in the urine might limit the numbers of bacteria at the attachment/colonization step of the pathogenic process. Small numbers of microorganisms might elicit a modest inflammatory response and induce antibodies specific for the particular strain. Second, in the non-immune non-secretor, colonization might not be inhibited but enhanced by interactions with Lewis a antigens as suggested by studies with candida [22,23] and by increased attachment of uropathogenic strains to cells of non-secretors [12]. The larger population of bacteria might multiply in the absence of specific immune protection and induce a strong inflammatory response. If this happened in the kidney, the inflammation might result in scar formation.

A role for antibodies in pathogenesis as well as protection can be postulated for urinary tract infections. In a rat lung model, binding of IgA-containing immune complexes to alveolar macrophages elicited production of reactive oxygen radicals resulting in significant damage to surrounding

tissues [24]. Similar reactions might occur in the bladder, contributing to the tissue damage and symptoms associated with these infections. If the immune responses in children parallel those we have observed in adults, we suggest that the increased levels of IgA in particular might contribute to renal scarring by mechanisms similar to that described for the rat lung model [6]. There are reports of local production of IgA in the urinary tract and significant levels of both IgA and IgG in the urine of children with pyelonephritis [25]. In the presence of complement, IgG containing complexes can elicit tissue damage via a similar mechanism via neutrophils [26].

Host–parasite interactions leading to establishment of urinary tract infections are multifactorial. Secretor status is a stable host marker that can be used to investigate the interactions between the host's innate and specific immune responses in both pathogenic and protective responses to infecting organisms.

ACKNOWLEDGEMENTS

We are grateful to Dr. J.A. Gray and Dr. P.D. Wellsby for access to their patients and for their support in this project, to Dr. M. Calder, Dr. B. Watt and the staff of the diagnostic laboratories of the City Hospital for their assistance, and to the Edinburgh and South-East Scotland Blood Transfusion Service for reagents used in the study. The Scottish project was supported by a grant from the Scottish Home and Health Department (K/MRS/50/C449) and by travel funds from the Wellcome Trust.

REFERENCES

[1] Asscher, A.W. (1982) A progress report on urinary tract infection, in Symposium on Renal Disease (Passmore, R., ed.), Royal College of Physicians, Edinburgh (publication No. 57) pp. 91–108.

[2] Kunin, C. (1970) A ten-year study of bacteriuria in school girls. Final report of bacteriological, urological and epidemiological findings. J. Infect. Dis. 122, 382–393.

[3] Rolleston, G.L., Maling, T.M.J. and Hodson, C.J. (1974) Intrarenal reflux and the scarred kidney. Arch. Dis. Child. 49, 531–539.

[4] Greenwood, B.M. (1984) Selective primary health care: strategies for control of disease in the developing world. XIII. Acute bacterial meningitis. Rev. Infect. Dis. 6, 374–389.

[5] Blackwell, C.C., May, S.J., Brettle, R.P., MacCallum, C.J. and Weir, D.M. (1986) Host-parasite interactions underlying non-secretion of blood group antigens and susceptibility to recurrent urinary tract infections, in Protein Carbohydrate Interactions in Biological Systems. (Lark, D., ed.), pp. 229–230, Academic Press, London.

[6] Blackwell, C.C., May, S.J., Brettle, R.P., MacCallum, C.J. and Weir, D.M. (1987) Secretor state and immunoglobulin levels among women with recurrent urinary tract infections. J. Clin. Lab. Immunol. 22, 133–137.

[7] Lomberg, H., Hellstrom, M., Jodal, U. and Svanborg-Eden, C. (1989) Secretor state and renal scarring in girls with recurrent pyelonephritis. FEMS Microbiol. Immunol. 47, 371–376.

[8] Kinane, D.F., Blackwell, C.C. Brettle, R.P., Weir, D.M., Winstanley, F.P. and Elton, R.A. (1982) ABO blood group, secretor state and susceptibility to recurrent urinary tract infections in women. Br. Med. J. 185, 7–9.

[9] Brettle, R.P., MacCallum, C.J., Murdoch, J.McC. and Gray, J.A. (1985) The adult female. A twenty year study of bacteriuria in female patients. In Microbial Diseases in Nephrology. (Brumfitt, W. and Asscher, A.W., eds.), pp. 257–271, John Wiley and Sons Ltd., New York.

[10] Smellie, J., Edwards, D., Hunter, N., Mormand, I.C.S. and Prescid, N. (1975) Vesicoureteric reflux and renal scarring. Kidney Int. (suppl. 4) 8, 565–572.

[11] May, S.J., Rahat, A., Blackwell, C.C., MacCallum, C.J., Brettle, R.P. and Weir, D.M. (1989) Characterization of *Escherichia coli* strains isolated from urine of secretors and non-secretors. FEMS Microbiol. Immunol. 47, 377–382.

[12] Lomberg, H., Cedergren, B., Leffler, H., Nilsson, B., Carlstrom, A.-S. and Svanborg-Eden, C. (1986) Influence of blood group antigens on the availability of receptors for attachment of uropathogenic *Escherichia coli*. Infect. Immun. 51, 919–926.

[13] Blackwell, C.C. (1989) The role of ABO blood groups and secretor status in host defences. FEMS Microbiol. Immunol. 47, 341–350.

[14] Blackwell, C.C., Weir, D.M., James, V.S., Cartwright, K.A.V., Stuart, J. and Jones, D.M. (1988) Non-secretion of ABO blood group antigens and susceptibility to infectious diseases, in Bacteria, Complement and the Phagocytic Cell. NATO ASI series (Cahello, F.C. and Prazzo, C., eds.), pp. 357–370, Springer Verlag, Heidelberg.

[15] Grundbacher, F.J. (1972) Immunoglobulins, secretor status and the incidence of rheumatic fever and rheumatic heart disease. Hum. Hered. 25, 399–404.

[16] Waissbluth, J.G. and Langman, J.S. (1971) ABO blood group, secretor status, salivary protein, and serum and salivary immunoglobulin concentrations. Gut 12, 646–649.

[17] Tuttle, J.P., Sarvas, H. and Koistinen, J. (1978) The role of vaginal immunoglobulin A in girls with recurrent urinary tract infections. J. Urol. 120, 742–744.

[18] Gotschlich, E.C., Goldschneider, I. and Artenstein, M.S. (1969) Human immunity to the meningococcus V. The effect of immunization with meningococcal group C polysaccharide on the carrier state. J. Exp. Med. 129, 1385–1395.

[19] Kalapothaki, V., Vassiliadis, P., Trichopoulos, D., Kaklamani, E. and Serie, Ch. (1979) Charactéristiques séro-epidémiologiques chez les porteur sains de méningocoques en Grèce. Ann. Microbiol. (Inst. Pasteur) 130 B, 43–54.

[20] Schonheyder, H., Johansen, J.A., Moller-Hansen, C. and Sterderup, A. (1983) IgA and IgG antibodies to *Candida albicans* in women of childbearing age. Sabouraudia 21, 223–231.

[21] Gough, P.M., Warnock, D.W., Richardson, M.D., Mansell, N.J. and King, J.M. (1984) IgA and IgG antibodies to *Candida albicans* in the genital tract secretions of women with or without vaginal candidiasis. Sabouraudia 22, 265–271.

[22] Thom, S.M., Blackwell, C.C., MacCallum, C.J., Weir, D.M., Brettle, R.P., Kinane, D.F. and Wray, D. (1989) Non-secretion of blood group antigens and susceptibility to infection by *Candida* species. FEMS Microbiol. Immunol. 47, 401–406.

[23] May, S.J., Blackwell, C.C. and Weir, D.M. (1989) Lewis ^{a} blood group antigen of non-secretors: a receptor for candida blastospores. FEMS Microbiol. Immunol. 47, 407–410.

[24] Johnson, K.J., Ward, P.A., Kunkel, R.G. and Wilson, B.S. (1986) Mediation of IgA induced lung injury in the rat. Lab. Invest. 54, 499–506.

[25] Sohl-Akerlund, A.-S., Ahlstedt, S., Hanson, L.A. and Jodal, U. (1979) Antibody responses in urine and serum against *Escherichia coli* O antigens in childhood urinary tract infections. Acta Pathol. Microbiol. Scand. C 87, 29–36.

[26] Johnson, K.J., Wilson, B.S., Till, G.O. and Ward, P.A. (1984) Acute lung injury in rat caused by IgA immune complexes. J. Clin. Invest. 74, 358–369.

[27] Nerell, G. (1963) Secretors of ABH antigens in a central Swedish population. Ann. Hum. Genet. Lond. 27, 119–123.

FEMS Microbiology Immunology 47 (1989) 377–382
Published by Elsevier

FIM 00062

Characterization of *Escherichia coli* strains isolated from urine of secretors and non-secretors

S.J. May, A. Rahat, C.C. Blackwell [1], C.J. MacCallum, R.P. Brettle [2] and D.M. Weir

[1] *Department of Bacteriology, The Medical School, University of Edinburgh, and* [2] *Pyelonephritis and Infectious Diseases Unit, The City Hospital, Edinburgh, U.K.*

Received 1 September 1988
Accepted 12 January 1989

Key words: Secretor; Non-secretor; Mannose-resistant haemagglutinin; Mannose-sensitive haemagglutinin; Haemolysin

1. SUMMARY

Strains of *Escherichia coli* isolated from urine of secretors (242) and non-secretors (121) were compared for their serotype and their ability to express mannose-sensitive (MS) haemagglutinins and mannose-resistant (MR) haemagglutinins and to produce haemolysin. The results of the survey refuted our hypothesis that strains with characteristics associated with virulence, those with MR haemagglutinins and/or haemolysins, would be isolated more frequently from non-secretors. MR haemagglutinins were detected among 36.4% of isolates from secretors and 27.3% of isolates from non-secretors. Haemolysin production was detected among 19.8% of isolates from secretors and 12.5% of isolates from non-secretors. Both MR haemagglutinins and haemolysin were detected only on 12.4% of strains from secretors and 6.7% of strains from non-secretors.

Correspondence to: C.C. Blackwell, Department of Bacteriology, The Medical School, University of Edinburgh, Edinburgh, U.K.

2. INTRODUCTION

The observations of increased proportions of non-secretors among women with recurrent urinary tract infections [1,2] and among young girls who developed kidney scars following urinary tract infection [3] has provided a stable host factor for investigation of the interactions between bacterial strains and epithelial cells that lead to colonization/infection. Mannose-resistant (MR) haemagglutinins are reported to be present on a high proportion of strains of *Escherichia coli* isolated from extraintestinal sites [4–6]. The report that strains with MR haemagglutinins bound in greater numbers to uroepithelial cells from non-secretors compared with uroepithelial cells from secretors [7] prompted us to compare expression of haemagglutinins among strains isolated from secretors and non-secretors. In this study we wished to examine 'disease markers' for both host and parasite to test the hypothesis that the more susceptible non-secretor hosts would be infected with the more virulent strains.

In vitro studies of haemagglutination inhibition of several adhesins associated with enteric patho-

gens suggested that oligosaccharides with blood group activity might be involved in blocking attachment of these bacteria to receptors on erythrocytes. Haemagglutination by CFA I, CFA II, and K88, fimbrial adhesins associated with enteropathogenic strains of *E. coli* is inhibited by oligosaccharides in pooled human milk, a rich source of secreted blood group antigens. This agglutination is not inhibited by mannose. Haemagglutination by type I fimbriae is inhibited by mannose (mannose-sensitive, MS), but not by these oligosaccharides from milk [8].

If secreted blood group antigens interact in a similar manner with the MR adhesins found on uropathogenic strains of *E. coli*, strains with these adhesins might more readily colonize non-secretors. In this study we characterized strains of *E. coli* from secretors and non-secretors for expression of MR and MS haemagglutinins, production of haemolysin and the serotype. A higher proportion of strains with MR haemagglutinins was predicted among the strains isolated from non-secretors but no difference in the proportion of strains with MS haemagglutinins was predicted.

3. SUBJECTS AND METHODS

E. coli strains were obtained from urine cultures examined by the diagnostic laboratory at the City Hospital, Edinburgh. They were subcultured to egg storage agar slopes and kept at room temperature until examined. MS haemagglutination was detected with guinea pig erythrocytes following culture in brain heart infusion (BHI) [9] and MR haemagglutination with human group A erythrocytes following incubation on CFA agar [10]. Haemolysin production was detected with horse blood agar and serotype by agglutination with commercially available antisera (Difco). Antisera to serotypes reported to be isolated commonly from urinary tract infections [11] were included in the set used: 01A, 01B, 02A, 02B, 04, 05, 06, 07, 08, 011, 012, 017, 018ab, 018ac, 020, 021, 022, 024, 025, 026, 045, 062, 068, 075, 086, 0111, 0140.

The 363 isolates were obtained from women attending outpatient clinics of the Infectious Diseases Unit, City Hospital, Edinburgh. ABO blood group of each patient was determined from a heparinized blood specimen by slide agglutination and secretor state from saliva by the haemagglutination inhibition assay [12].

The results were compared by the chi square test.

4. RESULTS

The results in Table 1 refute our prediction that strains expressing MR haemagglutinins would be isolated more frequently from infected urine of non-secretors. Although the proportion of strains with MR haemagglutinins is lower for non-secretors, the difference is not statistically significant. No difference in the proportion of strains with MS haemagglutinins was predicted; although the proportion of strains with MS haemagglutinins was lower among the isolates from non-secretors, the difference was not statistically significant (Table 2).

Haemolysin production was detected in 48 of the 242 strains (19.8%) isolated from secretors and in 15 of the 121 strains from non-secretors (12.5%). Among the strains isolated from secretors, 30 (12.4%) expressed both MR haemagglutinins and haemolysin. Among those isolated from non-secretors, 8 (6.7%) had both these characters. Although the proportions of strains with both these characteristics were lower among those isolated from non-secretors, the difference was not statistically significant.

The majority of strains obtained from these patients were either autoagglutinable or were not

Table 1

Proportion of strains obtained from secretors and non-secretors expressing haemagglutinins following incubation on CFA

	Mannose sensitive		Mannose resistant		None	
	no.	(%)	no.	(%)	no.	(%)
Secretors (242)	6	(2.5)	88	(36.4)	148	(61.1)
Non-secretors (121)	4	(3.3)	33	(27.3)	84	(69.4)

Table 2

Proportion of strains obtained from secretors and non-secretors expressing haemagglutinins following incubation in BHI

	Mannose sensitive		Mannose resistant		None	
	no.	(%)	no.	(%)	no.	(%)
Secretors (242)	102	(42.1)	15	(6.2)	125	(51.7)
Non-secretors (119)	43	(36.1)	3	(2.5)	73	(61.3)

agglutinated by any of the 28 antisera used in the study. Table 3 lists the numbers of strains agglutinated by the individual typing sera tested. A number of strains were polyagglutinable: 39 of the 242 strains from secretors (16%), and 18 of the 120 from non-secretors (15%) (Table 4).

There were 72 women from whom 2 or more isolates were obtained during the 5 years of the

Table 3

Agglutination of strains obtained from secretors and non-secretors

Serotype	Source of isolates			
	Secretors (242)		Non-secretors (120)	
	no.	%	no.	%
autoagglutinable	68	(28)	20	(16.7)
not agglutinable	61	(25)	44	(36.7)
1	14	(5.8)	6	(5)
2	4	(1.7)	1	(0.8)
4	5	(2.1)	1	(0.8)
5	3	(1.2)	1	(0.8)
6	9	(3.7)	0	0
7	16	(6.6)	7	(5.8)
8	3	(1.2)	0	0
11	3	(1.2)	1	(0.8)
12	3	(1.2)	1	(0.8)
17	14	(5.8)	6	(5)
18	7	(2.9)	2	(1.7)
21	2	(0.8)	2	(1.7)
22	9	(3.7)	4	(3.3)
24	12	(5)	8	(6.7)
25	7	(2.9)	7	(5.8)
26	9	(3.7)	2	(1.9)
45	16	(6.6)	5	(4.2)
62	2	(0.8)	0	0
68	2	(0.8)	1	(0.8)
75	7	(2.9)	8	(6.7)
78	3	(1.2)	1	(0.8)
86	0	0	1	(0.8)
111	1	(0.4)	0	0
140	3	(1.2)	4	(3.3)

Table 4

Polyagglutinable strains isolates from secretors and non-secretors

Agglutination pattern	Source of isolates			
	Secretors		Non-secretors	
	no. 39/242	(%) (16.1)	no. 18/120	(%) (15)
1,17,24	1		0	
1,2	1		0	
1,6	1		0	
2,5,26	1		0	
2,6,17	1		0	
4,18	1		0	
5,26	2		1	
6,7,17	1		0	
7,1,24	2		0	
7,22,24	1		0	
7,24	1		0	
7,4,26	1		0	
7,45	3		0	
7,45,26	1		1	
12,22	0		1	
12,25	1		0	
17,22	1		0	
17,24	1		4	
17,25	1		1	
17,45	1		0	
17,45,7	1		0	
18,4	0		1	
18,68	1		0	
18,86	0		1	
22,24	1		0	
24,17	1		0	
24,17,1	0		1	
24,18	1		0	
24,21	0		1	
24,25	1		1	
25,45	1		0	
25,78	0		1	
26,75	1		0	
45,140	1		0	
45,75	2		0	
62,75	2		0	
68,45	1		1	
75,45	1		0	
75,140	0		1	
111,26	1		0	
140,25	0		1	
140,75	0		1	

study. Of these 47 (28.8%) were secretors and 25 (28.7%) were non-secretors. Based on the properties used to characterize the isolates in the study, there were only 14 in which strains with identical properties were obtained on 2 or more occasions from the same patient. Of these, 9 patients were secretors and 5 non-secretors. MR haemagglutinins were detected on 3 of the strains from secretors but none of the strains from non-secretors expressed these adhesins. MS haemagglutinins were detected on 4 strains from secretors but only on 1 strain obtained from the non-secretors.

5. DISCUSSION

The results of this study did not substantiate our original hypothesis that we would find the strains with more virulence markers among the more susceptible non-secretors. The proportion of strains expressing either MR or MS haemagglutinins were lower among those isolated from non-secretors compared with those isolated from secretors. Haemolysin is an aggressin reported to be associated with pathogenic strains of *E. coli*, but, again, there were fewer strains from non-secretors producing this enzyme compared with strains from secretors.

These results suggest that there are adhesins or other virulence markers not yet identified that contribute to the pathogenesis of urinary tract infections. One hypothesis proposed to explain the increased proportion of non-secretors among patients with recurrent urinary tract infections is that the blood group antigens in body fluids of secretors can bind to adhesins on the surface of microorganisms, interfering with their colonization of epithelial cells [13]. Strains of *E. coli* were examined for their ability to adsorb A, B, and H antigens from body fluids by the haemagglutination method. Several of the clinical isolates adsorbed these antigens, principally H (Blackwell and Rahat, unpublished observations). The ability to adsorb H antigens was not dependent on the presence of MR or MS haemagglutinins or serotype.

Binding of *Streptococcus salivarius* to epithelial cells is inhibited by salivary glycoproteins with blood group activity [14], and binding of candida blastospores to epithelial cells is inhibited by preincubation of blastospores with boiled saliva of secretors but not that of non-secretors [15]. Studies are underway to determine if secreted blood group antigens might inhibit binding of uropathogenic strains to epithelial cells and to determine the nature of the components that adsorb the blood group antigens.

ACKNOWLEDGEMENTS

We are grateful to Dr. J.A. Gray and Dr. P.D. Wellsby for access to their patients and for their support for this study, to Dr. B. Watt, Dr. M. Calder and the staff of the diagnostic laboratory, City Hospital, Edinburgh for their cooperation. This work was supported by a grant from the Scottish Home and Health Department (K/MRS/C449) and by travel funds from the Wellcome Trust.

REFERENCES

[1] Kinane, D.F., Blackwell, C.C., Brettle, R.P., Weir, D.M., Winstanley, F.P. and Elton, R.A. (1982) ABO blood group, secretor state and susceptibility to recurrent urinary tract infections in women. Br. Med. J. 285, 7–9.

[2] Blackwell, C.C., May, S.J., Brettle, R.P., MacCallum, C.J. and Weir, D.M. (1987) Secretor state and immunoglobulin levels among women with recurrent urinary tract infections. J. Clin. Lab. Immunol. 22, 133–137.

[3] Lomberg, H., Hellstrom, M., Jordal, U. and Svanborg-Eden, C. (1989) Secretor state and renal scarring in girls with recurrent pyelonephritis. FEMS Microbiol. Immunol. 47, 371–376.

[4] Minshew, B.H., Jorgensen, J., Counts, G.W. and Falkow, S. (1978) Association of hemolysin production, haemagglutination of human erythrocytes, and virulence for chicken embryos of extra-intestinal *Escherichia coli* isolates. Infect. Immun. 20, 50–54.

[5] Evans, D.J., Evans, D.G., Young, L.S. and Pitt, J. (1980) Haemagglutination typing of *Escherichia coli*: Definition of seven hemagglutination types. J. Clin. Microbiol. 12, 235–242.

[6] Hagberg, L., Jodal, U., Korhonen, T.K., Lindin-Janson, G., Lindberg, U. and Svanborg-Eden, C. (1981) Adhesion,

haemagglutination and virulence of *Escherichia coli* causing urinary tract infections. Infect. Immun. 31, 564–570.

[7] Lomberg, H., Cedergren, B., Leffler, H., Nilsson, B., Carlstrom, A.-S. and Svanborg-Eden, C. (1986) Influence of blood group and availability of receptor for attachment of uropathogenic *Escherichia coli*. Infect. Immun. 51, 919–926.

[8] Holmgren, J., Svennerholm, A.-M. and Ahren, C. (1981) Nonimmunoglobulin fraction of human milk inhibits bacterial adhesion (hemagglutination) and enterotoxin binding of *Escherichia coli* and *Vibrio cholerae*. Infect. Immun. 33, 135–141.

[9] Svanborg-Eden, C. and Hanson, L.A. (1978) *Escherichia coli* pili as possible mediators of attachment to human urinary tract epithelial cells. Infect. Immun. 21, 229–237.

[10] Evans, D.G., Evans, D.J. and Tjoa, W. (1978) Haemagglutination of human group A erythrocytes of enterotoxigenic *Escherichia coli* isolated from adults with diarrhea: correlation with colonization factor. Infect. Immun. 18, 330–337.

[11] Ørskov, I., Ørskov, F., Birch-Anderson, A., Kanamoti, M. and Svanborg-Eden, C. (1982) O, K, H and fimbrial antigens in *Escherichia coli* serotypes associated with pyelonephritis and cystitis. Scand. J. Infect. Dis. (suppl) 33, 18–25.

[12] Mollison, P.L. (1979) Blood Transfusion in Clinical Medicine (6th edition) pp. 414–482. Blackwell Scientific Publications, Oxford.

[13] Blackwell, C.C. (1989) The role of ABO blood groups and secretor status in host defences. FEMS Microbiol. Immunol. 47, 341–350.

[14] Williams, R.C. and Gibbons, R.J. (1975) Inhibition of streptococcal attachment to receptors on human buccal epithelial cells by antigenically similar salivary glycoproteins. Infect. Immun. 11, 711–718.

FEMS Microbiology Immunology 47 (1989) 401-406
Published by Elsevier

FIM 00066

Non-secretion of blood group antigens and susceptibility to infection by *Candida* species

S.M. Thom [1], C.C. Blackwell [1], C.J. MacCallum [1], D.M. Weir [2], R.P. Brettle [1], D.F. Kinane [3] and D. Wray [1]

[1] *Department of Bacteriology and Department of Oral Medicine and Pathology, University of Edinburgh,* [2] *Division of Infectious Diseases, City Hospital, Edinburgh, and* [3] *Department of Periodontology, The Dental School, University of Dundee, Dundee, U.K.*

Received 1 September 1988
Accepted 12 January 1989

Key words: Blood group antigens; Secretor status; *Candida* infection

1. SUMMARY

One of the innate defences against superficial infections by *Candida* species appears to be the ability of an individual to secrete the water-soluble form of his ABO blood group antigens into body fluids. There was a significantly higher number of non-secretors (48.9%) among 174 patients with either oral or vaginal *candida* infections compared with the proportion of non-secretors in the local population (26.6%).

The protective effect afforded by the secretor gene might be due to the ability of glycocompounds in the body fluids of secretors to inhibit adhesins on the surface of the yeast. In attachment studies, preincubation of blastospores with boiled secretor saliva significantly reduced their ability to bind to epithelial cells. Non-secretor saliva did not reduce the binding and often enhanced the numbers of attached yeasts.

Possible host–parasite interactions underlying the susceptibility of non-secretors to *candida* and other infections are discussed.

Correspondence to: S.M. Thom, Department of Bacteriology, University of Edinburgh, Edinburgh, U.K.

2. INTRODUCTION

A 1980 review of the immune response to *Candida albicans* stressed the need to obtain more basic information on innate host defence mechanisms that control diseases caused by this opportunistic pathogen [1]. There were a number of observations that suggested non-secretion of blood group antigens might be associated with susceptibility to infection by the candida species. Williams and Gibbons [2] reported that salivary glycoproteins with blood group antigen activity were able to inhibit and even reverse binding of *Streptococcus salivarius* to buccal cells. If particular strains of *candida* have adhesins that recognize blood group antigens on host cells, we predicted interactions similar to those described for the streptococci might place on mucosal surfaces of secretors but not on those of non-secretors.

Fucose has been found to inhibit binding of the yeast to human epithelial cells [3]. Fucose is also the immunodominant sugar of the H antigen of blood group O and a terminal component of the Lewis antigens. Except for individuals of the extremely rare Bombay phenotype, the H antigen is present in body fluids of secretors of all four ABO blood groups; and, in 94% of secretors, there will

also be Lewis ^{a} antigen but predominantly Lewis ^{b} antigen [4].

This led to our hypothesis that the inhibition of binding of *candida* to epithelial cells by fucose might be due to inhibition of a lectin-like adhesin on the surface of the yeast. Glycoproteins on the surface of *candida* involved in binding have been isolated and characterized and one of these adhesins can be inhibited by fucose [5]. If these glycoproteins are involved in colonization, the H or Lewis ^{b} antigens of secretors might bind to the adhesin and decrease the probability of the yeast attaching to the host's mucosal surfaces.

The following questions arose from these proposals:

1. Is there an increase in the incidence of non-secretors among patients with candida infections?
2. Are there differences in binding of the yeast to epithelial cells if the blastospores are pre-incubated with saliva from secretors or non-secretors?

3. MATERIALS AND METHODS

Candida strains 3091 and 3118C originally isolated from oral infections were kindly provided by Dr. M.V. Martin, Department of Dental Sciences, The University of Liverpool. They were cultured on Sabouraud agar.

Saliva specimens were obtained from women attending outpatient clinics of the Infectious Diseases Unit (City Hospital, Edinburgh), from women attending ante-natal clinics (Royal Infirmary, Edinburgh), and from both men and women attending dental outpatient clinics for investigation of oral *candida* infections (the dental schools University of Edinburgh and University of Dundee).

Secretor state was determined by the haemagglutination inhibition method described by Mollison [6] and the results compared with those for local blood donors [7].

Attachment of the yeasts to buccal epithelial cells was determined microscopically. In order to eliminate the possibility of contamination by secreted blood group antigens, buccal cells were obtained from non-secretor donors of blood groups A, B, and O. Two donors of each group were used. Saliva samples obtained from secretors and non-secretors of blood groups A, B and O were boiled for 30 min in glass universal containers to inactivate enzymes that destroy secreted blood group antigen and any antibodies present. They were centrifuged to remove particulate matter and stored at $-20\,^{\circ}C$. The yeasts were incubated with saliva samples or Dulbecco's phosphate buffered saline containing supplement B composed of Ca^{2+} and Mg^{2+} (0.9 mM and 0.5 mM respectively) (DPBS + B). Mixtures of yeasts and buccal epithelial cells from a non-secretor of the corresponding ABO blood group (10 yeasts per buccal cell) were incubated for 1 h at $37\,^{\circ}C$. Mixtures were centrifuged and washed 3 times to remove non-adherent blastospores and slides prepared in a cytospin. The slides were Gram-stained and 60 cells per slide observed for numbers of attached yeasts.

The results were analyzed by Wilcoxon's rank sums test as described by Swinscow [8]. The analysis of Rosenstein et al. [9] suggested results of attachment studies have a non-parametric distribution and that transformation of the data for application of more exact tests was not valid. They recommended using the medians rather than the means for calculation of the P values. In these studies we found no difference between the P values when either the means or the medians were analyzed by this test.

4. RESULTS

4.1. Epidemiological studies

The numbers of non-secretors for the different patient groups are compared with figures for local blood donors, 26.6% [7]. Of the 174 patients, 48.9% ($\chi^2 = 24.069$, $P < 0.0005$) were non-secretors.

The proportion of non-secretors among 101 women with a history of recurrent *candida* infections was 40.6% ($\chi^2 = 6.549$, $P < 0.025$). There were also 30 pregnant women with symptoms of infection and from whom *candida* was isolated; among these 17 (56.7%; $\chi^2 = 10.608$, $P < 0.005$) were non-secretors.

Of the 43 patients, both male and female, referred to the dental hospitals for treatment of recurrent oral *candida* infections, 27 (62.8%) were non-secretors ($\chi^2 = 21.697$, $P < 0.0005$).

4.2. Attachment of blastospores to buccal epithelial cells

To test our hypothesis that binding of blastospores might be inhibited by heat-stable glycocompounds in the body fluids of secretors, we compared attachment of blastospores of the two *candida* strains following preincubation of the yeasts in either DPBS + B or boiled saliva from secretor donors. If a lectin-like interaction is involved in attachment, the supplement B of the DPBS supplied the divalent cations needed. The general pattern observed was that, in comparison with the controls incubated with DPBS + B, incubation of the blastospores of either strain with secretor saliva resulted in reduction of the numbers of yeasts attached to the buccal cells. This pattern was observed with cells and saliva from donors of blood groups A, B, and O and with both strains of *candida*. Analysis by Wilcoxon rank sum test of 10 experiments with 3091 and 13 experiments with 3118C found preincubation of the blastospores with secretor saliva significantly reduced their ability to bind to epithelial cells ($P < 0.05$).

Saliva from non-secretors did not have a similar effect and often increased the attachment of yeasts to the buccal cells. Analysis by Wilcoxon rank sum test of 8 experiments comparing binding of blastospores preincubated in DPBS + B with that of blastospores preincubated in non-secretor saliva found a significant increase in binding in the presence of non-secretor saliva ($P < 0.01$).

Dilution of the secretor saliva reduced its ability to inhibit binding of *candida* to the epithelial cells. The inhibitory effect was not present at dilutions in which there was no detectable blood group antigen and was usually lost by dilutions of 1 in 10.

5. DISCUSSION

As predicted, we found a significantly higher proportion (54.4%) of non-secretors among patients with symptomatic *candida* infections or individuals who had a history of recurrent *candida* infections compared with that found for the local population (26.6%) [7]. When the patient groups were analyzed, the significant increases in the numbers of non-secretors were found among those who were immunocompromised by pregnancy or those who had a history of recurrent infections.

Our recent studies of patients with infections due to *Neisseria meningitidis*, *Streptococcus pneumoniae* and *Haemophilus influenzae* revealed significant increases in the proportions of non-secretors among these individuals [10,11]. The compromising factor among these patients is known. At the time of exposure to the particular pathogen, they lacked the serogroup specific antibodies that protect the host from invasive disease [12]. The specific immune responses that protect the host from *candida* infections are not yet defined. Identification of non-secretion as a stable host factor that predisposes the non-immune individual to these infections provides a basis for investigation of the host-parasite interactions involved.

The results of the attachment experiments support our hypothesis that heat-stable components in the body fluids of secretors might inhibit binding of an adhesin on the yeast. Boiled saliva from secretors inhibited attachment of the blastospores of both 3091 and 3118c to buccal epithelial cells. The controls for the attachment studies, the numbers of blastospores per cell in the absence of saliva, varied with each experiment. Inhibition also varied with saliva from different donors. This might be due to the varying amounts of blood group antigens present in the saliva of secretors of the different ABO groups [13] or the conditions under which the saliva was collected, e.g., stimulated specimens have less blood group antigen than unstimulated specimens [14–16].

Our epidemiological observations of a higher proportion of non-secretors among patients with invasive pneumococcal and haemophilus infections were complemented by those of Andersson and coworkers [17] who found that glycocompounds from pooled human milk were able to inhibit binding of these pathogens to epithelial cells. The Lewis ^{b}, and H antigens common to the body fluids of most secretors are candidates for the inhibitory substance(s). Recent work from our laboratory suggested that Lewis ^{b} is not the component responsible for inhibition of the two strains tested [18,19].

Table 1

Proportion of non-secretors among patients with infection due to *Candida albicans*

Category	Total	Non-secretor		χ^2	P
		no.	(%)		
Controls *	334	89	(26.6)		
Total patients	174	85	(48.9)	24.069	< 0.0005
Genital infection pregnant history of recurrent infection	30	17	(56.7)	10.608	< 0.005
	101	41	(40.6)	6.549	< 0.025
Oral infection	43	27	(62.8)	21.697	< 0.0005

* local blood donors.

The enhanced binding observed with non-secretor saliva was an unexpected finding. It helps to explain the report that a number of the fresh saliva specimens from which specific anti-*candida* antibodies had been adsorbed increased the binding of the yeast to epithelial cells [20]. This phenomenon was found with approximately 25% of their donors, the expected proportion of non-secretors in an unselected population.

Binding of microorganisms to epithelial cells [21] and phagocytic cells [22,23] by lectin-like interactions has been reported by a number of groups. Although there is no direct evidence as yet that the carbohydrate moieties of ABO or Lewis blood groups act as receptors for microorganisms, we are investigating the possible role of these antigens as receptors not only for *candida* but also for other microorganisms.

The high proportion of non-secretors among immunocompromised individuals with *candida* infections suggests that if specific immunity to these pathogens is absent or transiently reduced, the ability to secrete the ABO antigens plays a role in the innate defences of the host against these opportunistic infections. Identification of non-secretors among immunocompromised patients such as those with antibody to HIV might help identify those at particular risk of superficial *candida* infections and perhaps other opportunistic infections.

ACKNOWLEDGEMENTS

This work was supported by a grant from Glaxo Group Research, Ltd. We are grateful to the Edinburgh and South-East Scotland Blood Transfusion Service for reagents used to determine secretor state and to Dr. F.D. Johnstone, Department of Obstetrics and Gynaecology, Royal Infirmary, Edinburgh for clinical specimens.

REFERENCES

[1] Rogers, T.J. and Balish, E. (1980) Immunity to *Candida albicans*. Microbiol. Rev. 44, 660–682.

[2] Williams, R.C. and Gibbons, R.J. (1975) Inhibition of streptococcal attachment to receptors on human buccal epithelial cells by antigenically similar salivary glycoproteins. Infect. Immun. 11, 711–718.

[3] Sobel, J.D., Myers, P.G., Kaye, D. and Lawson, M.E. (1981) Adherence of *Candida albicans* to human vaginal and buccal epithelial cells. J. Infect. Dis. 143, 76–82.

[4] Boorman, K.E., Dodd, B.E. and Lincoln, P.J. (1977) Blood Group Serology. 5th ed. pp. 161–190, Churchill Livingstone, Edinburgh.

[5] Critchley, I.A. and Douglas, L.J. (1987) Role of glycoproteins as epithelial cell receptors for *Candida albicans*. J. Gen. Microbiol. 133, 637–643.

[6] Mollison, P.L. (1979) Blood Transfusion in Clinical Medicine. 6th edition. pp. 414–482, Blackwell Scientific Publications, Oxford.

[7] Kinane, D.F., Blackwell, C.C., Brettle, R.P., Weir, D.M. Winstanley, F.P. and Elton, R.A. (1982) ABO blood group, secretor state and susceptibility to recurrent urinary tract infections in women. Br. Med. J. 285, 7–9.

[8] Swinscow, T.D.V. (1985) Statistics at Square One. pp. 58–61. The Mendip Press, Bath.

[9] Rosenstein, R.J., Grady, D., Hamilton-Miller, J.M.T. and Brumfitt, W. (1985) Relationship between adhesion of *Escherichia coli* to uroepithelial cells and the pathogenicity of urinary tract infection: problems in methodology and analysis. J. Med. Microbiol. 20, 335–344.

[10] Blackwell, C.C., Jonsdottir, K., Hanson, M., Todd, W.T.A., Chaudhuri, A.K.R., Mathew, B., Brettle, R.P. and Weir, D.M. (1986a) Non-secretion of ABO antigens predisposing to infection by *Neisseria meningitidis* and *Streptococcus pneumoniae*. Lancet ii, 284–285.

[11] Blackwell, C.C., Jonsdottir, K., Weir, D.M. and Hansson, M.F. (1986b) Non-secretion of ABO antigens predisposing to infection by *Haemophilus influenzae*. Lancet ii, 687.

[12] Greenwood, B.M. (1984) Selective primary health care: strategies for control of disease in the developing world. XIII. Acute bacterial meningitis. Rev. Inf. Dis. 6, 374–378.

[13] Race, R.R. and Sanger, R. (1975) Blood Groups in Man. 6th edition, pp. 311–322, Blackwell Scientific Publications. Oxford.

[14] Milne, R.W. and Dawes, C. (1973) The relative contribution of blood-group substance in the parotid, sublingual and submaxillary salivas. Vox Sang. 25, 298–307.

[15] Waissbluth, J.G. and Langman, M.J.S. (1971) ABO blood groups secretor status, salivary protein, and serum and salivary immunoglobulin concentrations. Gut 12, 646–649.

[16] Wolf, R.O. and Taylor, L.L. (1964) The concentration of blood group substance in the parotid, sublingual and submaxillary salivas. J. Dent. Res. 43, 272–275.

[17] Andersson, B., Porras, O., Hanson, L.A., Lagergard, T. and Svanborg-Eden, C. (1986) Inhibition of attachment of *Streptococcus pneumoniae* and *Haemophilus influenzae* by human milk and receptor oligosaccharides. J. Infect. Dis. 153, 232–237.

[18] May, S.J., Blackwell, C.C. and Weir, D.M. (1986) Non-secretion of blood group antigens and susceptibility to *Candida albicans:* the role of Lewis blood group antigens. J. Dent. Res. 65, 503.

[19] May, S.J., Blackwell, C.C. and Weir, D.M. (1989) Lewis ^{a} blood group antigen of non-secretors: a receptor for candida blastospores. FEMS Microbiol. Immunol. 47, 407–410.

[20] Epstein, J.B., Kimura, L.H., Menard, T.W., Truelove, E.L. and Pearsall, N.N. (1982) Effects of specific antibodies on the interaction between the fungus *Candida albicans* and human oral mucosa. Arch. Oral Biol. 27, 469–474.

[21] Beachey, E.A., Eisenstein, B.I. and Ofek, I. (1983) Bacterial adherence in infectious diseases, in Current Concepts, pp. 3–52, The Upjohn Company, Kalamazoo, Michigan.

[22] Weir, D.M. (1980) Surface carbohydrates and lectins in cellular recognition. Immunol. Today. 1, 45–51.

[23] Kinane, D.F., Weir, D.M., Blackwell, C.C. and Winstanley, F.P. (1984) Binding of *Neisseria gonorrhoeae* by lectin-like receptors on human phagocytic cells. J. Clin. Lab. Immunol. 13, 107–110.

FEMS Microbiology Immunology 47 (1989) 407–410
Published by Elsevier

FIM 00067

Lewis [a] blood group antigen of non-secretors: a receptor for *candida* blastospores

S.J. May, C.C. Blackwell and D.M. Weir

Department of Bacteriology, The Medical School, University of Edinburgh, Edinburgh, U.K.

Received 1 September 1988
Accepted 12 January 1989

Key words: Lewis [a]; Blood group antigen; *Candida* receptor

1. SUMMARY

This study tested the hypothesis that the Lewis [a] blood group antigen found predominantly on the cells of non-secretors might be one of the receptors for *Candida* species. Binding of strain 3118C to epithelial cells from either secretor or non-secretor donors was not inhibited by treating the cells with anti-Lewis [a] or anti-Lewis [b] antisera. Binding of strain 3091 to non-secretor cells was inhibited by pretreating the cells with anti-Lewis [a], but this was not observed for secretor cells.

The results suggest that Lewis [a] might be one of the receptors for some yeast strains.

2. INTRODUCTION

In our epidemiological studies of patients with either oral or vaginal infections due to *Candida* species we have reported a significant increase in the proportion of individuals who are non-secretors of the ABO blood group antigens [1–3]. Here we present evidence for one of the hypotheses proposed to explain the host-parasite interactions underlying these observations, that the Lewis [a] antigen is one of the receptors for pathogenic strains of *candida*.

Glycoproteins that mediate attachment of candida blastospores to epithelial cells have been described. Binding of various strains by these adhesins can be inhibited by monosaccharides, some by fucose and others by *N*-acetylglucosamine [4]. Since fucose is the immunodominant sugar of the Lewis [a] antigen, we predicted that pretreatment of epithelial cells of non-secretors might inhibit binding of some strains of *candida*.

If this proposal is correct, the ability of the Lewis antigens to adsorb on to the cell surface might explain our observation that pre-treatment of blastospores with non-secretor saliva enhanced their binding [1–3]

3. MATERIALS AND METHODS

Candida strains 3091 and 3118C originally isolated from patients with oral infections were kindly provided by Dr. M.V. Martin, Liverpool University. They were maintained on egg storage slopes at room temperature. Overnight cultures grown on Sabouraud's agar were used to prepare suspen-

Correspondence to: S.J. May, Department of Bacteriology, The Medical School, University of Edinburgh, Edinburgh, U.K.

sions of blastospores (2×10^6 ml). Buccal epithelial cells (BEC) were obtained from a secretor and a non-secretor, both of blood group B. The BEC were washed once in Dulbecco's phosphate buffered saline plus supplement B containing calcium and magnesium ions (0.9 mM and 0.5 mM respectively) (DPBS + B). They were divided into 3 aliquots and an equal volume of DPBS + B (control), anti-Lewis ^{a} or anti-Lewis ^{b} antisera (Behring) added. After incubation at 37° C for 1 h, the cells were washed 3 times by centrifugation ($400 \times g$ for 10 min) with DPBS + B and adjusted to a final concentration of 2×10^5 ml.

The attachment assay was performed by a modification of the method described by Kimura and Pearsall [5]. Duplicate samples of each control and test were prepared by mixing equal volumes (100 μl/ml) of the BEC and the blastospores. After incubation at 37° C for 60 min in an orbital incubator (100 rpm), the mixtures were washed 3 times by centrifugation at $400 \times g$ and resuspended in 50 μl of fresh DPBS + B. Slides were prepared with 20 μl of each sample, air-dried, fixed with methanol and Gram-stained. The numbers of adherent yeasts on 60 individual cells were counted on each slide.

The results of these experiments were compared by Wilcoxon's rank sum test [6]. In view of the recommendation of Rosenstein et al. [7], the results obtained with both the means and medians were compared in these analyses.

4. RESULTS

Table 1 summarizes the results of 9 experiments with BEC obtained from a secretor donor and Table 2 the results of 9 experiments with BEC obtained from a non-secretor donor. The same statistical values were obtained with either the means or the medians. Treatment of BEC from either donor with anti-Lewis ^{b} antiserum did not alter the attachment of either strain, 3091 or 3118C, compared with the control. Treatment of

Table 1

Attachment of strains 3091 and 3118C to secretor buccal epithelial cells (BEC) pre-treated with anti-Lewis ^{a} or anti-Lewis ^{b} antisera

Serotype	Mean * number of yeasts per BEC following pre-treatment of secretor BEC					
	DPBS	anti-Le ^{a}	*P*-value +	DPBS	anti-Le ^{b}	*P*-value +
3091	2.09	2.22	NS	2.09	2.13	NS
3118C	2.61	1.94	NS	2.61	2.53	NS

DPBS, Dulbecco's PBS + B supplement.
Le ^{a}, Lewis ^{a}; Le ^{b}, Lewis ^{b}.
* Mean number of yeasts per BEC from 9 experiments.
+ Wilcoxon's Rank Sum Test; NS, not significant.

Table 2

Attachment of strains 3091 and 3118C to non-secretor buccal epithelial cells (BEC) pre-treated with anti-Lewis ^{a} or anti-Lewis ^{b} antisera

Serotype	Mean * number of yeasts per BEC following pre-treatment of non-secretor BEC					
	DPBS	anti-Le ^{a}	*P*-value +	DPBS	anti-Le ^{b}	*P*-value +
3091	2.48	1.69	< 0.05	2.48	1.91	NS
3118C	1.96	1.95	NS	1.96	1.95	NS

DPBS, Dulbecco's PBS + supplement.
Le ^{a}, Lewis ^{a}; Le ^{b}, Lewis ^{b}.
* Mean number of yeasts per BEC from 9 experiments.
+ Wilcoxon's Rank Sum Test; NS, not significant.

BEC from the non-secretor donor with anti-Lewis ^{a} antiserum significantly reduced the binding of strain 3091 but not strain 3118C blastospores compared with the control. Treatment of the BEC from the secretor donor with anti-Lewis ^{a} antiserum did not alter the attachment of either strain.

5. DISCUSSION

Significant inhibition of binding of the blastospores to BEC was observed only for non-secretor cells pre-treated with anti-Lewis ^{a} antisera. These results were obtained only with 3091, indicating that there might be an adhesin on this strain that can bind to the Lewis ^{a} antigen.

Lewis ^{b} does not appear to be the receptor for either strain tested. This suggests that it is not the component in secretor saliva that reduces binding of the blastospores to epithelial cells [3]. The H antigen is a possible candidate for the inhibitory substance, but we have been unable to demonstrate adsorption of the H antigen from secretor saliva by haemagglutination inhibition assays for either 3091 or 3118C or 10 other strains of candida examined (MacLean and Blackwell, unpublished observations).

Unlike the ABO antigens, the Lewis antigens are not structural components of the host cells. They are adsorbed on to the cells from body fluids. If a lectin-like adhesin on blastospores can recognize Lewis ^{a} antigens in the body fluids of non-secretors, the following interactions might occur. The yeast could bind directly to the Lewis ^{a} antigen on the surface of an epithelial cell. Alternatively, the carbohydrate moiety of the Lewis ^{a} antigen in secretions could bind to the adhesin on the blastospore and then become attached to an epithelial cell by the normal adsorption processes. This model explains our observations that pre-treatment of blastospores with non-secretor saliva increased binding of the yeast compared with controls pre-treated with DPBS + B.

Susceptibility to superficial *candida* infections is multifactorial as is protection against these opportunistic pathogens. Our results suggest that the ability to secrete ABO blood group antigens is one of the host's innate defence mechanisms. The non-secretor is at a disadvantage in two respects: (1) absence of the substance(s) in secretor saliva that inhibit binding of the blastospores of some strains to epithelial cells and (2) presence of an antigen on his cells that is a receptor for the adhesins on some strains of *candida* blastospores.

ACKNOWLEDGEMENT

This work was supported by a grant from the Wellcome Trust.

REFERENCES

[1] Blackwell, C.C., Thom, S.M., Lawrie, O.R., Weir, D.M., Wray, D. and Kinane, D.F. (1986) Non-secretion of blood group antigens and susceptibility to oral infections by *Candida albicans*. J. Dent. Res. 65, 502.

[2] Blackwell, C.C., Thom, S.M., Weir, D.M., Kinane, D.F. and Johnstone, F.D. (1986) Host-parasite interactions underlying non-secretion of blood group antigens and susceptibility to infections by *Candida albicans*, in Protein-Carbohydrate Interactions in Biological Systems. (Lark, D., ed.), pp. 231–233, Academic Press, London.

[3] Thom, S.M., Blackwell, C.C., MacCallum, C.J., Weir, D.M., Brettle, R.P., Kinane, D.F. and Wray, D. (1989) Non-secretion of blood group antigens and susceptibility to infection by *Candida* species. FEMS Microbiol Immunol. 47, 401–406.

[4] Critchley, I.A. and Douglas, L.J. (1987) Role of glycoproteins as epithelial cell receptors for *Candida albicans*. J. Gen. Microbiol. 133, 637–643.

[5] Kimura, L.H. and Pearsall, N.N. (1978) Adherence of *Candida albicans* to human buccal epithelial cells. Infect. Immun. 21, 64–68.

[6] Sinscow, T.D.V. (1985) Statistics at Square One. pp. 58–61, The Mendip Press, Bath.

[7] Rosenstein, R.J., Grady, D., Hamilton-Miller, J.M.T. and Brumfitt, W. (1985) Relationship between adhesion of *Escherichia coli* to epithelial cell and the pathogenicity of urinary tract infection: problems in methodology and analysis. J. Med. Microbiol. 20, 335–344.

FEMS Microbiology Immunology 47 (1989) 351–356
Published by Elsevier

FIM 00058

Blood group, secretor status and susceptibility to bacterial meningitis

C.C. Blackwell [1], K. Jonsdottir [2], D.M. Weir [1], M.F. Hanson [3], K.A.V. Cartwright [4], J. Stewart [5], D. Jones [6] and I. Mohammed [7]

[1] *Department of Bacteriology, the Medical School, University of Edinburgh, Edinburgh, U.K.,* [2] *Department of Bacteriology, University of Iceland, Reykjavik, Iceland,* [3] *Central Microbiology Laboratory, Edinburgh,* [4] *Public Health Laboratory, Gloucester,* [5] *Community Medicine, Gloucester,* [6] *Public Health Laboratory, Manchester, U.K. and* [7] *Department of Medicine, University of Maiduguri, Maiduguri, Nigeria*

Received 1 September 1988
Accepted 12 January 1989

Key words: ABO blood groups, Meningococci; Meningitis, bacterial; Non-secretion; Preunococci; Type b haemophilus

1. SUMMARY

Epidemiological evidence is summarized for associations of ABO blood group and secretor status with susceptibility to invasive disease due to capsulate organisms responsible for the majority of bacterial meningitis. Host-parasite interactions that might underly these findings are proposed and evidence to support or refute them provided.

2. INTRODUCTION

Bacterial meningitis is still a major health problem in both western and developing nations. In the United Kingdom at least 1 child in every 1000 develops acute bacterial meningitis by the age of 10 [1]. Approximately 75% of all these infections are caused by three species: *Neisseria meningitidis*, *Haemophilus influenzae* and *Streptococcus pneumoniae*. Each of these organisms has in common polysaccharide capsules that appear to play a role in pathogenesis. Protection against invasive disease caused by these strains is associated with the presence of opsonizing or bactericidal antibodies specific for the invading strain and an intact complement system. The natural development of antibodies to these organisms is reflected in the age range in which these infections are most prevalent, 6 months to about 4.5 years. These are infants and young children in whom protective maternal antibody has waned and who are not yet able to produce an effective long-term response to polysaccharide antigens [1].

Of all the diseases for which an association with secretor state has been observed, bacterial meningitis has provided the clearest model on which to test several of the suggested hypotheses. Regardless of age, the compromises in the specific immune defences of the host against these organisms is well defined. The majority of individuals with these infections lack protective antibodies to the invading pathogen, and a minority have

Correspondence to: C.C. Blackwell, Department of Bacteriology, The Medical School, University of Edinburgh, Edinburgh, U.K.

congenital defects in either their complement or properdin systems [2,3]. The genetic factors that predispose the non-immune individual to resistance or susceptibility to these diseases are only beginning to be elucidated. There are small numbers of rare individuals in whom complement components are absent or less efficient, but more common genetic markers, ABO blood group and secretor status, appear to be associated with susceptibility to these infections.

3. ABO BLOOD GROUPS

Reed et al. [4] found that among 88 patients with pneumococcal disease and 16 carriers of the organism there was a significantly lower proportion of group B individuals. They suggested that the anti-A isohaemagglutinins of B individuals were able to act as natural antibodies against these bacteria. Their studies revealed that pneumococci could acquire either A or B antigens if the appropriate red blood cells were present in the growth medium. In the same paper they reported that 5 of 7 strains tested expressed antigens that cross-reacted with A antigen even if the bacteria were grown on medium containing B blood. In vitro assays with lysed bacteria demonstrated an enzyme capable of altering B antigen to the A-like antigen.

They interpreted their epidemiological and laboratory findings as follows. Some strains of pneumococci can incorporate A antigens from tissues and fluids of group A hosts, but the host lacks anti-A antibodies. Group O blood endows most strains with little if any substances that will cross-react with A or B antigens. Consequently, the anti-A and anti-B isohaemagglutinins of group O individuals seldom or never act as natural antibodies for these strains. Individuals of group AB lack both isohaemagglutinins, so protective humoral defences among individuals of blood groups A, O and AB depend on specific anti-pneumococcal antibodies. The low proportion of group B individuals with these infections is dependent on (1) the presence of anti-A antibodies, (2) the ability of the invading strain to convert B antigens to A antigens and (3) the ability to incorporate this cross-reactive substance into the bacterial cell envelope.

The congruence of the meningitis belt of central Africa with the largest area in which there is an unusually high proportion of the population with the blood group B gene is a clue that we are pursuing with colleagues in northern Nigeria [5]. Although the B gene is found in about 25% of the population in the area studied, there does not appear to be a higher proportion of individuals with the B gene among the 40 patients with meningococcal infection so far examined [6].

4. SECRETOR STATUS

Epidemiological evidence from studies in the United Kingdom, Iceland and Nigeria suggest that there is an association between non-secretion and susceptibility to invasive disease caused by meningococci, pneumococci and haemophilus [6–8] (Table 1). In most western European populations, the proportion of non-secretors is 20–25% [9]. In areas where there have been prolonged outbreaks of meningococcal disease and where the secretor status of the population has been determined (northern Nigeria and Iceland), there are unusually high proportions of non-secretors. A similar pattern was found in Stonehouse, Gloucestershire which was investigated during a prolonged outbreak of disease due to a serogroup B serotype 15 : P1.16 sulphonamide resistant (B15R) strain of meningococci. The proportion of non-secretors in Stonehouse (32.7%) was significantly higher than that of the surrounding area (23.4%) [10].

4.1. The role of secretor status in host defences against bacterial meningitis

We have examined several of the hypotheses suggested to explain how secretor status might effect both innate defences such as the initial colonization of the host by these organisms and the immune responses to these bacteria.

4.2. Secretor status and carriage

The significant increase in the proportion of non-secretors among patients with these infections suggested a parallel with rheumatic fever epide-

Table 1

Proportion of non-secretors among patients with invasive infections due to *N. meningitidis*, *H. influenzae* type b and *Strep. pneumoniae*

Organism	Total	Non-secretors		*P*
		No.	(%)	
N. meningitidis				
United Kingdom				
Scotland				
Controls	334	89	(26.6)	< 0.005
Patients	26	18	(69)	
Stonehouse				
Controls	277	134	(32.7)	
Patients	13	7	(54)	
Iceland				
Controls	228	92	(41.2)	< 0.05
Patients	98	53	(54)	
Nigeria				
Controls	186	92	(49.5)	< 0.01
Patients	42	31	(73.3)	
H. influenzae				
Iceland				
Controls	228	94	(41.2)	< 0.005
Patients	43	29	(67)	
Strep. pneumoniae				
Scotland				
Controls	334	89	(26.6)	< 0.01
Patients	47	22	(47)	

miology. Non-secretors were found to be over-represented among patients with rheumatic fever and also among individuals who were carriers of the group A *Streptococcus pyogenes* [11]. The recent study of meningococcal carriage in Stonehouse provided an opportunity to examine this hypothesis [12].

There was not an increased proportion of non-secretors among carriers of the B15R meningococci. Of the 67 residents who were carriers of the outbreak strain, 20 (30%) were non-secretors. Among 459 carriers of meningococci other than the outbreak strain, 132 (29%) were non-secretors. During a particular period of time, non-secretors do not appear to be more likely to be carriers than secretors. The effect of secretor status on long-term carriage has not yet been assessed [10].

Carriage of meningococci can be influenced by the presence of serum antibodies specific for the capsular antigen. Immunization of recruits with group C polysaccharide vaccine reduced carriage of serogroup C but not other serogroups among the immunized population [13]; and, among Greek recruits, presence of antibodies to serogroup B in an individual at the time of induction reduced the probability of becoming a carrier [14]. The information regarding the specific antibody levels of carriers and controls to the B15R strain is not complete, so the question regarding influence of secretor status on carriage in the non-immune host is not yet answered.

4.3. The IgA levels of secretors and non-secretors

Non-secretors have been reported to have lower levels of both salivary [15] and serum IgA [16]. Since IgA is thought to play a role in reducing colonization by pathogens, the lower levels of salivary IgA reported for non-secretors [15] suggested specific immune responses at the mucosal surfaces of non-secretors might be compromised compared with those of secretors. Material collected in the Stonehouse survey allowed us to test this hypothesis. Our examination of salivary IgA levels with commercially available immunodiffusion plates did not confirm these earlier observations. There was no difference in the mean levels of IgA in the saliva of 165 secretors (2.1 IU/ml) compared with 109 non-secretors (1.9 IU/ml) from whom no neisseriae were isolated. Compared with non-carriers, carriers of meningococci, 168 secretors (3.2 IU/ml) and 92 non-secretors (3.7 IU/ml), had significantly raised levels of salivary IgA [17]. Both secretor and non-secretor carriers of *Neisseria lactamica* had salivary IgA levels comparable to those of non-carriers (Fig. 1).

In acute sera, antibodies to capsular polysaccharides of meningococcal serogroups A and C and to type b haemophilus are predominantly IgA [18]. Purified IgA from patients with infections due to meningococcal serogroups B, Y, or C inhibited complement mediated bacteriolysis by IgG and IgM isotypes [19]. Purified IgG and IgM antibodies to type b *H. influenzae* capsular polysaccharide are opsonic and bactericidal, but IgA antibodies to this antigen are not [20].

In an epidemiological study of children in Gambia during an outbreak of meningococcal disease, measurement of total immunoglobulin levels showed that 'children who subsequently became

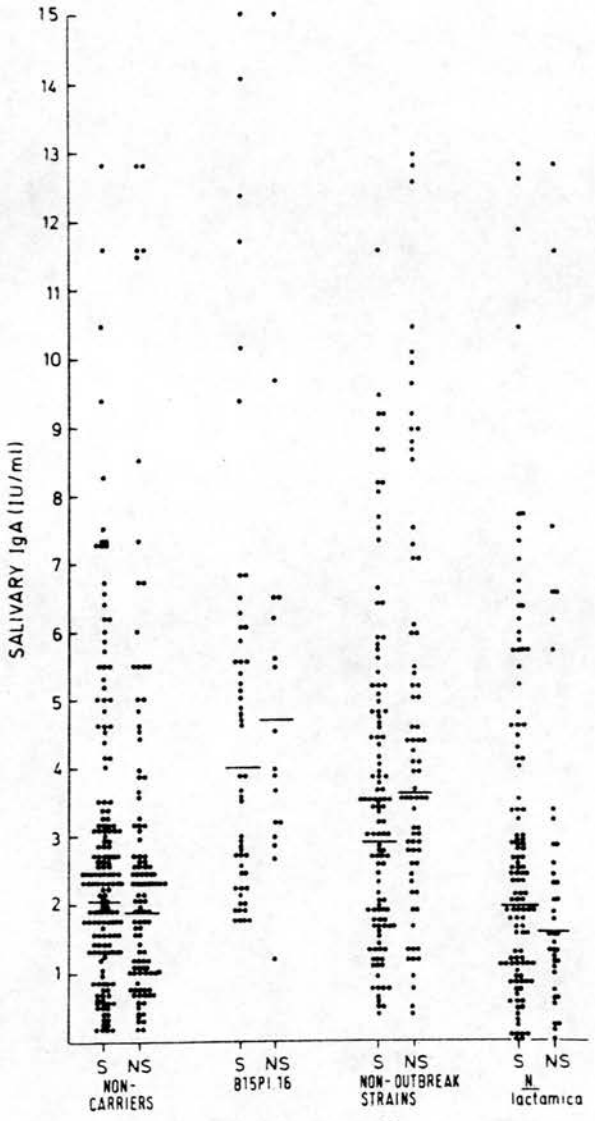


Fig. 1. Salivary IgA levels of secretors and non-secretors among non-carriers, carriers of *N. meningitidis* and carriers of *N. lactamica*.

cases had a higher mean IgA value than did controls' [21]. We had found higher levels of total serum IgA among non-secretor women with recurrent UTI [22]. If total immunoglobulin levels partly reflect antibodies specific for the colonizing strain, these increased levels of IgA antibodies among non-secretors might effectively compete with the complement fixing isotypes for binding sites on the invading bacteria.

We were able to confirm the early report of lower levels of serum IgA among non-secretors [16]. Among Stonehouse residents from whom no neisseria were isolated the mean serum IgA levels for 148 secretors was 115 IU/ml which was significantly higher than that for 102 non-secretors, 102 IU/ml ($P < 0.025$). Compared with the secretor control, there was no significant difference in the mean serum IgA of 101 secretors carrying serogroupable strains (116 IU/ml) or 66 carriers of non-serogroupable strains (119 IU/ml). Compared with non-secretor controls, 50 non-secretors carrying serogroupable strains had a significantly higher mean serum IgA level (130 IU/ml) ($P < 0.0025$), but the difference between the non-carriers and the 32 carriers of non-serogroupable strains (85 IU/ml) was not significant.

The immune response of the host has been implicated to play a role in the pathogenesis of these infections [19]. In view of the possible role of IgA to polysaccharide antigens acting as blocking antibodies competing with the complement fixing isotypes and subclasses, the immune responses of secretors and non-secretors to these capsulate organisms need to be compared. Studies of the immune responses of secretors and non-secretors to polysaccharide vaccines and polysaccharide–protein conjugates might provide information on possible problems associated with their development.

4.4. Predisposing infections and secretor status

It has been suggested that viral and mycoplasma infections might act as predisposing factors for bacterial meningitis [23]. Pneumococcal pneumonia following influenza is a classic example of viral infection predisposing humans to invasive disease due to capsulate bacteria, and a similar pattern has been found for invasive disease due to *Haemophilus influenzae suis* following swine influenza in pigs [24]. Although there are several reports of associations between ABO blood groups and susceptibility to natural or experimental viral infections [25–27], there is no published evidence that non-secretion is associated with susceptibility to viral infections. It is, however, a line of investigation that might provide new insights into the pathogenesis of meningococcal disease.

5. APPLICATION OF THESE STUDIES

Protection against several serogroups of these bacteria can be elicited by immunization of adults and older children with capsular polysaccharide antigens. The protection elicited is specific only for the serogroup from which the polysaccharide was prepared; and, in infants and very young children, these have not been found to be effective immunogens. The increase in the proportion of non-secretors is found among patients with meningococcal disease due to each of the serogroups A, B and C and also among patients with invasive disease due to *Str. pneumoniae* and type b haemophilus. By determining the host–parasite interactions responsible for this increased susceptibility of non-secretors, we might identify a new approach to prevention of bacterial meningitis that circumvents the problems associated with capsular polysaccharide antigens and the narrow spectrum of serotype-specific vaccines.

ACKNOWLEDGEMENT

This work was supported by a grant from the Meningitis Research Trust.

REFERENCES

[1] Greenwood, B.M. (1984) Selective primary health care: strategies for control of disease in the developing world. XII. Acute bacterial meningitis. Rev. Infect. Dis. 6, 374–389.

[2] Ross, S.C. and Densen, P. (1984) Complement deficiency states and infection: epidemiology, pathogenesis and consequences of neisserial and other infections in an immune deficiency. Medicine 63, 243–273.

[3] Densen, P., Weiler, J.M., Griffiss, J.M. and Hoffman, L.G. (1987) Familial properdin deficiency and fatal meningococcemia: correction of the bactericidal defect by vaccination. N. Engl. J. Med. 316, 922–931.

[4] Reed, W.F., Drach, G.W. and Williams, R.C. (1974) Antigens common to human and bacterial cells. IV. Studies of human pneumococcal disease. J. Lab. Clin. Med. 83, 599–610.

[5] Winstanley, F.P., Blackwell, C.C. and Weir, D.M. (1985) Factors influencing susceptibility to meningococcal disease. Biomed. Pharmacother. 39, 167–170.

[6] Blackwell, C.C., Jonsdottir, K., Mohammed, I., and Weir, D.M. (1988) Non-secretion of blood group antigens — a genetic factor predisposing to infection by *Neisseria meningitidis*, in Gonococci and Meningococci (J.T. Poolman et al., eds.), pp. 633–636, Kluwer Academic Publishers, Dordrecht.

[7] Blackwell, C.C., Jonsdottir, K., Hanson, M., Todd, W.T.A., Chaudhuri, A.K.R., Mathew, B., Brettle, R.P. and Weir, D.M. (1986) Non-secretion of ABO antigens predisposing to infection by *Neisseria meningitidis* and *Streptococcus pneumoniae*. Lancet ii, 284–285.

[8] Blackwell, C.C., Jonsdottir, K., Hanson, M.F. and Weir, D.M. (1986) Non-secretion of ABO antigens predisposing to infection by *Haemophilus influenzae*. Lancet ii, 687.

[9] Eriksson, A.W., Partanen, K., Frants, R., Pronk, J.C. and Kostense, P.J. (1986) ABH secretion polymorphism in Icelanders, Aland islanders, Finns, Finnish Lapps, Komi and Greenland Eskimos: a review and new data. Ann. Hum. Biol. 13, 273–285.

[10] Blackwell, C.C., Weir, D.M., James, V.S., Cartwright, K.A.V., Stuart, J.M. and Jones, D.M. (1989) The Stonehouse study: secretor status and carriage of *Neisseria* species Epidemiol. Infect. 102, 1–10.

[11] Haverkorn, M.J. and Goslings, W.R.O. (1979) Streptococci, ABO blood groups and secretor status. Am. J. Hum. Genet. 21, 360–375.

[12] Stuart, J.M., Cartwright, K.A.V., Jones, D.M., Noah, N.D. Wall, R.J., Blackwell, C.C., Jephcott, A.E. and Ferguson, I.R. (1987) An outbreak of meningococcal disease in Stonehouse: planning and execution of a large scale survey. Epidemiol. Infect. 99, 579–589.

[13] Gotschlich, E.C., Goldschneider, I. and Artenstein, M.S. (1969) Human immunity to the meningococcus. V. The effect of immunization with meningococcal group C polysaccharide on the carrier state. J. Exp. Med. 129, 1385–1395.

[14] Kalapothaki, V., Vassiliadis, P., Trichopoulos, D., Kaklamani, E. and Serie, Ch. (1979) Characteristiques sero-epidemiologiques chez les porteurs sains de méningocoques en Gréce. Ann. Microbiol. (Inst. Pasteur) 130 B, 43–54.

[15] Waissbluth, J.G. and Langman, J.S. (1971) ABO blood groups, secretor status, salivary protein, and serum and salivary immunoglobulin concentrations. Gut 12, 646–649.

[16] Grundbacher, F.J. (1972) Immunoglobulins, secretor status and the incidence of rheumatic heart disease. Hum. Hered. 25, 399–404.

[17] Blackwell, C.C., Weir, D.M., James, V.S., Cartwright, K.A.V., Stuart, J.M. and Jones, D.M. (1989) Non-secretion of ABO blood group antigens and susceptibility to infectious diseases, in Bacteria, Complement and the Phagocytic cell. NATO ASI series (Cahello, F.C. and Prazzo, C., eds.), pp. 357–370, Springer Verlag, Heidelberg.

[18] Kayhty, H., Jousimies-Somer, H., Peltola, H. and Makela, P.H. (1981) Antibody response to capsular polysaccharides of group A and C *Neisseria meningitidis* and *Haemophilus influenzae* type b during bacteremic disease. J. Infect. Dis. 143, 32–41.

[19] Griffiss, J.M. (1975) Bactericidal activity of meningococcal antisera: blocking by IgA of lytic antibody in human convalescent sera. J. Immunol. 114, 1779–1784.

[20] Schreiber, J.R., Barrus, V., Cates, K.L. and Siber, G.R. (1986) Functional characterization of human IgG, IgM and IgA antibody directed to the capsule of *Haemophilus influenzae* type b. J. Infect. Dis. 153, 8–16.

[21] Greenwood, B.M., Greenwood, A.M., Gradley, A.K., Williams, K., Hassan-King, M., Shenton, F.C., Wall, R.A. and Hayes, R.J. (1987) Factors influencing susceptibility to meningococcal disease during an epidemic in the Gambia. J. Infect. 14, 167–184.

[22] Blackwell, C.C., May, S.J., Brettle, R.P., MacCallum, C.J. and Weir, D.M. (1987) Secretor state and immunoglobulin levels among women with recurrent urinary tract infections. J. Clin. Lab. Immunol. 22, 133–137.

[23] Krasinski, K., Nelson, J.D., Butler, S., Luby, J.P. and Kusmiesz, H. (1987) Possible association of mycoplasma and viral respiratory infections with bacterial meningitis. Am. J. Epidemiol. 125, 499–508.

[24] Shope, R.E. (1944) Old, intermediate and contemporary contributions to our knowledge of pandemic influenza. Medicine 23, 415.

[25] McDonald, J.C. and Zukerman, A.J. (1962) ABO blood groups and acute respiratory virus disease. Br. Med. J. 11, 89–90.

[26] Potter, C.W. (1969) Haemagglutination inhibition antibody to various influenza viruses and adenoviruses in individuals of blood groups A and O. J. Hyg. (Camb.) 67, 67–74.

[27] Mackenzie, J.S. and Fimmel, P.J. (1978) The effect of ABO blood groups on the incidence of epidemic influenza and on the response to live attenuated and detergent split influenza vaccines. J. Hyg. (Camb.) 80, 21–30.

Diabetes Research (1989) 10, 000–000

37
DIABETES RESEARCH

BLOOD GROUP, SECRETOR STATUS AND ORAL CARRIAGE OF YEASTS AMONG PATIENTS WITH DIABETES MELLITUS

C. C. BLACKWELL,[1] F. Z. M. ALY,[1] V. S. JAMES,[1] D. M. WEIR,[1] A. COLLIER,[2] A. W. PATRICK,[2] C. G. CUMMING,[3] D. WRAY[3] and B. F. CLARKE[2]

[1]Department of Bacteriology, The Medical School, University of Edinburgh, [2]Department of Diabetic and Dietetics, Royal Infirmary, Edinburgh and [3]Department of Oral Medicine and Oral Pathology, The Dental School, University of Edinburgh, UK
(Received 5 May 1989)

SUMMARY The inability to secrete the water-soluble glycoprotein form of the ABO blood group antigens is a genetic characteristic associated with susceptibility to superficial fungal infections and also insulin-dependent diabetes mellitus (IDDM). As oral carriage of *Candida albicans* in healthy adults is associated with non-secretion, we examined oral carriage of yeasts among 275 patients attending diabetic outpatient clinics, 137 with IDDM and 138 with non-insulin dependent diabetes mellitus (NIDDM) with reference to ABO blood group, secretor status and yeast species.

Of the 166 yeast isolates, 109 (66·7%) were *C. albicans*, a lower proportion compared with 94% reported for healthy individuals. There was no association between ABO blood group and carriage. There was no increase in the proportion of non-secretor carriers of *C. albicans* among patients with IDDM: but among those with NIDDM, 44% of non-secretors were carriers compared with 21% who were non-carriers ($p < 0{\cdot}01$). The results are discussed in the context of host-parasite interactions influencing colonization.

Key words: Insulin-dependent diabetes mellitus, non-insulin dependent diabetes mellitus, *Candida albicans*, non-secretion

INTRODUCTION

EXAMINATION of genetic factors associated with susceptibility to infectious agents is providing new insights into the host–parasite interactions that result in disease. One of these characteristics, non-secretion of the ABO blood groups antigens, has been associated with susceptibility to a number of infectious diseases (1–6) and some autoimmune diseases (including insulin dependent diabetes mellitus) for which infectious triggers have been postulated (7–10). The carbohydrate antigenic determinants of ABO blood groups are found in two forms: glycolipids that are part of the structure of the host cell: and water-soluble glycoproteins that are present in body fluids such as saliva and urine. The ability to secrete the glycoprotein form is controlled by a single gene on chromosome 19. The majority of individuals in western European populations are secretors (75–80%), while the minority (20–25%) express the recessive non-secretor phenotype (11, 12).

One hypothesis suggested to explain the increased susceptibility of non-secretors is that they are more readily colonized by some pathogenic species. Epidemiological evidence for this hypothesis was summarized for patients with rheumatic fever by Haverkorn and Goslings (2). There is a significantly higher proportion of non-secretors among patients with rheumatic fever and also among carriers of the causative organism, group A *Streptococcus pyogenes*. Carriage of *Candida albicans* among healthy subjects has been found to be significantly associated with blood group O and, independently, with non-secretion of the ABO blood group antigens (13).

Our earlier studies have found a significant increase in the proportion of non-secretors among non-diabetic patients with recurrent oral or vaginal candida infections (14). As *C. albicans*, often causes oral or vaginal infections among patients with diabetes, we examined a group of 275 patients with diabetes for oral carriage of these fungi, ABO blood group and secretor status to determine if there were similar associations between candida carriage, blood group O and non-secretion reported for healthy individuals.

Correspondence: Dr. C. C. Blackwell, Department of Bacteriology, The Medical School, University of Edinburgh, UK.

0265-5985/89/040000-0000 $00.00

Subjects and Methods

Patients (275) attending routine out-patient clinics participated in the study. None of the patients was admitted to hospital or being treated for superficial fungal infections. Patients who had insulin dependent diabetes (IDDM) were classified by insulin dependence, clinical history and family history of the disease. Those with non-insulin dependent diabetes (NIDDM) were not dependent on insulin.

ABO and Lewis groups were determined by agglutination of heparinized blood samples and the secretor status by the haemagglutination inhibition assay from saliva samples (15).

A quantitative mouthwash technique was used to examine carriage of candida. The participants swilled 10 ml of sterile saline in their mouths for 1 min and returned the wash to a sterile container. The sample was processed within 2 hr of collection. Each specimen was concentrated by centrifugation and resuspension of the pellet in 1 ml sterile saline: 0·02 ml of the material was inoculated on malt agar plates. The plates were incubated at 37°C for 48 hr in an atmosphere of 5–10% CO_2. The colonies were counted and the species determined by germ tube production and API Candida (API 20C).

In order to compare our results for patients with IDDM or NIDDM with those reported for healthy individuals, the definition of Burford-Mason *et al.* (13) for non-carrier was used. Non-carrier status was assigned to a patient if no yeast colonies were isolated from the quantitative mouthwash.

The results were analyzed by the X^2 test.

Results

No yeast was isolated from 115 (42%) of the 275 patients examined, but one or more species of yeast was obtained from 160 (58%) patients. Yeasts were isolated from 84 (61%) of the 137 patients with IDDM and 76 (55%) of the 138 patients with NIDDM. Among patients with IDDM, the proportion of carriers was 65% for secretors and 56% for non-secretors. Among those with NIDDM, 49% of the secretors were carriers as were 68% of non-secretors. These differences in the proportions of non-secretors in the carrier and non-carrier groups were not statistically significant (Table 1).

Table 1 Carriage of yeasts among secretors and non-secretors with IDDM or NIDDM

	IDDM		*NIDDM*	
	Carrier no. (%)	*Non-carrier no. (%)*	*Carrier no. (%)*	*Non-carrier no. (%)*
Secretor	54 (65)	29 (35)	48 (49)	49 (51)
Non-secretor	30 (56)	24 (44)	28 (68)	13 (32)
Total	84 (61)	53 (39)	76 (55)	62 (45)

Table 3 Secretor status of carriers of *C. albicans* compared with that of non-carriers

	IDDM		*NIDDM*	
	Secretor no. (%)	*Non-secretor no. (%)*	*Secretor no. (%)*	*Non-secretor no. (%)*
Non-carriers	29 (55)	24 (45)	49 (79)	13 (21)*
C. albicans	38 (67)	19 (33)	29 (56)	23 (44)*

* $p < 0.01$.

More than one species was isolated from six of the patients. Of the 166 isolates, 109 (65·7%) were *C. albicans*, and the remaining 57 (34·3%) were predominantly other strains of *Candida* or *Torulopsis*; six were not identifiable (Table 2).

Examination of the data for patients with IDDM found no significant differences in the proportions of non-secretors among individuals from whom no yeast was isolated (45%) compared with carriers of *C. albicans* (33%). Although the proportion of secretors with IDDM carrying *C. albicans* was higher (67%) than secretors from whom no yeasts were isolated (55%), the difference was not statistically significant (Table 3).

Among patients with NIDDM there was a significant increase in the proportion of non-secretors from whom *C. albicans* was isolated (44%) compared with non-secretors who were non-carriers (21%) ($X^2 = 6·7$, $p < 0·01$); however, there was no significant difference in the proportions of secretors who were carriers of *C. albicans* (56%) compared with secretors who were non-carriers (79%) (Table 3).

There was no significant difference in the distribution of A or O blood groups between patients with diabetes who were carriers compared with non-carriers of *C. albicans*. There were not enough patients of blood group B or AB for statistical analysis (Table 4). Among patients with NIDDM there was, however, a significantly higher proportion of non-secretors among group O carriers of *C. albicans* (13 26, 50%) compared with the proportion of non-secretors among group O patients from whom no yeasts were isolated (8 36, 22%) ($p < 0·05$). A similar pattern was observed for blood group A, 6 of the 15 carriers (40%)

Table 2 Yeasts isolated from secretors and non-secretors with IDDM or NIDDM

	IDDM			*NIDDM*		
	Total	*Secretor no. (%)*	*Non-secretor no. (%)*	*Total*	*Secretor no. (%)*	*Non-secretor no. (%)*
Yeasts	86	55 (64)	31 (36)	80	50 (63)	30 (34)
C. albicans	57	38 (67)	19 (33)	52	29 (56)	23 (44)
Others	29	17 (59)	12 (41)	28	21 (75)	7 (25)

Table 4. Distribution of O and A blood groups among carriers and non-carriers of *C. albicans*

	Total	O no. (%)	A no. (%)
IDDM			
Non-carriers	44	26 (59)	18 (41)
C. albicans	54	31 (57)	23 (43)
NIDDM			
Non-carriers	54	36 (67)	18 (33)
C. albicans	41	26 (63)	15 (37)
Blood donors	277	173 (62)	104 (38)

were non-secretors but only 3 of the 19 non-carriers (16%). The numbers are too small for analysis by the X^2 method. This pattern was not observed among patients with IDDM.

Discussion

The proportion of 275 diabetics from whom yeasts were isolated (58%) is similar to that reported in other studies (16, 17) and higher than that for healthy controls (30%) (13). There was a higher proportion of carriers among non-secretors (58/95, 61%) compared with secretors who were carriers (102/180, 55%) but this was not statistically significant (Table 1).

Compared with healthy controls, there were differences in the species of yeasts isolated from patients with diabetes: 94% of those from healthy subjects were *C. albicans* (13) compared with 65·7% from diabetics. The remaining 34·3% of isolates from diabetics were mainly other species of *Candida* and *Torulopsis*. The lower proportion of *C. albicans* and an increase in the more unusual species of yeasts parallel the pattern found among individuals with antibody to the human immunodeficiency virus (HIV) (Wray, unpublished observations). This might reflect the susceptibility of immunocompromised individuals to organisms that are not usually carried by or pathogenic in the healthy host.

Analysis of the data by type of diabetes yielded results that do not agree with the report by Lamey *et al.* (17). They found no association between non-secretion and carriage of candida among 58 patients with IDDM or 50 with NIDDM: however, the numbers of patients in their study were less than half the number examined here and there was no differentiation of *C. albicans* from other species. Comparison of their results with ours or those of Burford-Mason and colleagues is difficult.

Analysis of the figures for carriers of *C. albicans* with non-carriers was performed to determine if there was a pattern similar to that obtained for healthy controls (13). Although there was no significant difference in the proportion of secretors or non-secretors with IDDM who were carriers, there was a significant increase in the proportion of non-secretors with NIDDM who were carriers of *C. albicans* (Table 3).

In contrast to the results of Burford-Mason *et al.* (13), we found no association between carriage of *C. albicans* and blood group O among patients with IDDM or NIDDM. There was, however, a significantly higher proportion of O non-secretors among the NIDDM carriers compared with those of blood group O who were not carriers. A similar pattern was observed among patients of blood group A with NIDDM, but the difference was not statistically significant. This pattern was not observed for patients with IDDM.

This study suggests an association between carriage of *C. albicans* and non-secretion similar to that reported for healthy subjects is found among patients with NIDDM but not among those with IDDM. The host-parasite interactions that influence carriage in the non-diabetic individuals appear to be similar to those in patients with NIDDM. The lower proportion of non-secretors among patients with IDDM who were carriers of *C. albicans* was unexpected and might be explained by investigation of their specific immune responses to these organisms.

In a group of women with recurrent urinary tract infections who were followed for 20 yr, non-secretors had significantly higher levels of total serum IgA and IgG than secretors. Improvement, judged as fewer episodes of infection during the second decade compared with the number during the first decade of the study, was associated with higher levels of total serum IgA in the non-secretors. We suggested this might reflect a greater dependency of non-secretors on specific immune responses for protection against these infections (18).

Decreased isolation of *C. albicans* from vaginal specimens is associated with high levels of specific anti-candida serum IgA, but not anti-candida serum IgG (19). Studies of the specific humoral immune responses of patients with NIDDM or IDDM to *C. albicans* are underway to determine if these differ between the two patient groups and what role these responses play in colonization with respect to secretor status.

ACKNOWLEDGEMENTS

This work was supported by a grant from the Wellcome Trust. We are grateful to the patients attending the Department of Diabetes and Dietetics for their cooperation in this study and to Mrs. M. K. Cole for assistance in preparation of the manuscript.

REFERENCES

1. Chaudhuri, A. and Das Adhikary, C. R. (1978). Possible role of blood group secretory substances in the aetiology of cholera. *Trans. Roy. Soc. Trop. Med. H.*, 72, 664–665.
2. Haverkorn, M. J. and Goslings, W. R. O. (1969). Streptococci, ABO blood groups and secretor status. *Am. J. Hum. Genet.*, 21, 360–375.
3. Kinane, D. F., Blackwell, C. C., Brettle, R. P., Weir, D. M.,

Winstanley, F. P. and Elton, R. A. (1982). ABO blood group, secretor state and susceptibility to recurrent urinary tract infection in women. *Br. Med. J.*, **285**, 7–9.

4. Blackwell, C. C., Thom, S. M., Lawrie, O., Weir, D. M., Wray, D. and Kinane, D. F. (1986). Non-secretion of blood group antigens and susceptibility to oral infections due to *Candida albicans*. *J. Dent. Res.*, **65**, 502.
5. Blackwell, C. C., Jonsdottir, K., Hanson, M., Todd, W. T. A., Chaudhuri, A. K. R., Mathew, B., Brettle, R. P. and Weir, D. M. (1986). Non-secretion of ABO blood group antigens predisposing to infection by *Neisseria meningitidis* and *Streptococcus pneumoniae*. *Lancet*, **ii**, 284–285.
6. Blackwell, C. C., Jonsdottir, K., Hanson, M. F. and Weir, D. M. (1986). Non-secretion of ABO blood group antigens predisposing to infection by *Haemophilus influenza*. *Lancet*, **ii**, 687.
7. Andersen, J. and Lauritzen, E. (1960). Blood groups and diabetes mellitus. *Diabetes*, **9**, 20–24.
8. Blackwell, C. C., James, V. S., Weir, D. M., Gemmill, J. D., Patrick, A. W., Collier, A. and Clarke, B. F. (1987). Secretor state of patients with insulin dependent or non-insulin dependent diabetes mellitus. *Br. Med. J.*, **295**, 1024–1025.
9. Shinebaum, R., Blackwell, C. C., Forster, P. J. G., Hurst, N. P., Weir, D. M. and Nuki, G. (1989). Non-secretion of ABO blood group antigens as a host susceptibility factor in the spondylarthropathies. *Br. Med. J.*, **294**, 208–210.
10. Collier, A., Patrick, A. W., Toft, A. D., Blackwell, C. C., James, V. S. and Weir, D. M. (1988). Increased prevalence of non-secretors in patients with Graves' disease—evidence for an infective aetiology. *Br. Med. J.*, **296**, 1162.
11. Mourant, A. E., Kopec, A. C. and Domaniewska-Sobczak, K. (1976). *The Distribution of the Human Blood Groups and Other Polymorphisms*. Oxford University Press, London.
12. Erikson, A. W., Partanen, K., Frants, R. R., Pronk, J. C. and Kostense, P. J. (1986). ABH secretion polymorphism in Icelanders, Aland islanders, Finns, Finnish Lapps, Komi and Greenland Eskimos: a review and new data. *Ann. Hum. Biol.*, **13**, 273–285.
13. Burford-Mason, A. P., Welsby, J. C. P. and Willoughby, J. M. T. (1988). Oral carriage of *Candida albicans*, ABO blood group and secretor status in healthy individuals. *J. Med. Vet. Mycology*, **26**, 49–56.
14. Thom, S. M., Blackwell, C. C., MacCallum, C. J., Weir, D. M., Brettle, R. P., Kinane, D. F. and Wray, D. (0000). Non-secretion of ABO blood group antigens and susceptibility to infection by *Candida* species. *FEMS Microbiol. Immunol.* ~~in press~~ 47 : 401-410 1990
15. Mollinson, P. L. (1979). *Blood Transfusion in Clinical Medicine* (sixth edition). Blackwell Scientific Publications, Oxford.
16. Weinstein, I. W., Duke, L. B., Peters, R. S. and Bahn, A. N. (1960). *Candida albicans* in the saliva of diabetics. *J. Dent. Res.*, **30**, ~~650-652~~ 6501
17. Lamey, P. J., Darwaza, A., Fisher, B. M., Samaranayake, L. P., MacFarlane, T. W. and Frier, B. M. (1988). Secretor status, candidal carriage and candida infection in patients with diabetes mellitus. *J. Oral Pathol.*, **17**, 354–357.
18. Blackwell, C. C., May, S. J., Brettle, R. P., MacCallum, C. J. and Weir, D. M. (1987). Secretor state and immunoglobulin levels among women with recurrent urinary tract infections. *J. Clin. Lab. Immunol.*, **22**, 133–137.
19. Schonneyder, N., Johansen, J. A., Moller-Hansen, C. and Sterderup, A. (1983). IgA and IgG serum antibodies to *Candida albicans* in women of childbearing age. *Sabouraudia*, **21**, 223–231.

Secretor State and Susceptibility to Recurrent Urinary Tract Infections

C. C. Blackwell, S. J. May, C. J. Maccallum, R. P. Brettle, and D. M. Weir

From the Department of Bacteriology, The Medical School, University of Edinburgh, and the Infectious Disease Unit, City Hospital, Edinburgh, Scotland

The inability to secrete the water-soluble form of the ABO blood group antigens is associated with susceptibility to a number of bacterial and fungal infections and to recurrent urinary tract infections (UTI) in women and appears to influence development of kidney scarring in young children. Three hypotheses are proposed to explain these observations, and evidence is provided to support or refute these suggestions. The hypothesis was tested that increased susceptibility of nonsecretors to recurrent UTI might be due in part to their reported lower levels of serum and secretory IgA. In contrast, among women observed for 20 years, the total serum IgA and IgG levels of 70 nonsecretors (125.4 and 139.7 IU/mL, respectively) were significantly higher than those for 111 secretors (103 and 118 IU/mL, respectively). IgA levels for both secretors and nonsecretors were significantly higher than those for 100 age-matched women in a prospective study group. Nonsecretors who had improved (fewer episodes of infection during the second decade of study) had significantly higher levels of both IgA ($P < .0025$) and IgG ($P < .025$) than did secretors who had improved. The possible role of the immune response of nonsecretors in both protection from reinfection and pathogenic sequelae of UTI is discussed.

Although a great deal of information has been obtained regarding the genetics and molecular biology of the uropathogenic *Escherichia coli* strains, information regarding stable susceptibility factors of the host is much more limited. It has been suggested that the secretor state of the host might be associated with susceptibility or resistance to infectious agents [1, 2]. The ability to secrete the water-soluble glycoprotein forms of ABO blood group antigens is a stable characteristic controlled by a single gene (*Se*) inherited in a Mendelian dominant pattern. The recessive, nonsecretor phenotype is expected in 25% of an outbred population; however, this figure can vary with different ethnic groups [3–5]. Secretor state does not alter with changes in the environment or age.

Our research group and other workers have found significantly higher proportions of nonsecretors among patients with cholera [6], superficial candida infections [7, 8], meningococcal meningitis, invasive pneumococcal disease [9], invasive infections due to type b *Haemophilus influenzae* [10], recurrent urinary tract infections (UTI) [11–13], and two diseases for which infectious etiologies have been proposed, ankylosing spondylitis [14] and type 1 diabetes mellitus [15].

Although a number of these observations were made in the 1960s, these epidemiologic findings of association between secretor state, ABO blood groups, and infectious diseases were not used as a basis for investigation of the underlying host-parasite interactions. The only hypothesis suggested at the time was that the isohaemagglutinins might act as "natural" antibodies against microorganisms with surface antigens cross-reactive with A or B. The recognition of lectin interactions between host and parasite provided the basis for a number of new hypotheses.

The secretor gene is involved not only in the secretion of A, B, and H antigens into body fluids but also with the expression of the Lewis blood groups [16]. The distribution of ABO and Lewis antigens in body fluids is shown in table 1. The body fluids of secretors (e.g., urine and saliva) will contain the ABO antigens of the individual, a small amount of Lewis a antigen, and a much greater proportion of Lewis b antigen. The body fluids of nonsecretors contain only Lewis a antigens. These differences suggested the first two of the three hypotheses we proposed to explain the host-parasite interaction under-

This project was supported by a grant from the Scottish Home and Health Department (K/MRS/50/C449).

We are grateful to Dr. J. A. Gray and Dr. P. Welsby for access to their patients and for their support in these studies, to Dr. M. Calder and Dr. B. Watt and the staff of the diagnostic laboratories of the City Hospital for their assistance, and to the Edinburgh and South-East Scotland Blood Transfusion Service for reagents used in this study.

Please address requests for reprints to Dr. C. C. Blackwell, Department of Bacteriology, The Medical School, Edinburgh EH8 9AG, United Kingdom.

Table 1. Distribution of the glycoprotein forms of ABO and Lewis antigens in body fluids of secretors and nonsecretors.

	ABO and Lewis antigens				
	A	B	H	Le^a	Le^b
Secretor	+/0*	+/0*	+	±†	+
Nonsecretor	0	0	0	+	0

* Dependent on presence of A or B gene.
† Some Le^a present in secretors.

lying the increased susceptibility of nonsecretors to infections (table 2).

Hypothesis 1: The inhibition hypothesis. In 1975, Williams and Gibbons reported that salivary glycoprotein with blood group activity were able to inhibit and even reverse binding of *Streptococcus salivarius* to epithelial cells [17]. A similar effect has been observed by Holmgren and co-workers for glycocompounds in pooled human milk, a rich source of blood-group antigens. In vitro these heat-stable compounds were able to inhibit adhesins on enteropathogenic strains of *E. coli* and *Vibrio cholerae*. They found that mannose-resistant but not mannose-sensitive binding was inhibited by these substances [18].

We predicted that the ABO or Lewis b antigens in body fluids of secretors might be able to inhibit binding of microbial adhesins. In attachment studies with *Candida*, we were able to demonstrate inhibition of binding of the yeast to epithelial cells with boiled saliva from secretors but not with saliva from nonsecretors [7, 8]. Andersson and co-workers have reported inhibition of binding of *Streptococcus pneumoniae* and *H. influenzae* by preincubating the bacteria with heat-stable glycocompounds from pooled human milk [19]. Their findings complement our epidemiologic studies in which we found an increased incidence of nonsecretors among patients with diseases caused by these bacteria [9, 10]. Our recent studies have found that several strains of *E. coli* isolated from infected urine can adsorb H antigen from body fluids (C. C. Blackwell, unpublished observations). If interactions similar to those in the in vitro experiments occur in vivo, secretors might be less easily colonized if the glycocompounds in their body fluids are able to inhibit lectin-like adhesins on the surface of the bacteria.

Hypothesis 2: The receptor hypothesis. The second hypothesis suggested that the Lewis a antigen is one of the receptors for uropathogenic strains. There is indirect evidence for this from three sources. Lomberg and co-workers have reported a significant increase in binding of strains with the mannose-resistant adhesins that recognize the α-D-Gal-(1→4)-β-D-Gal conformation (mannose-resistant [MR]:globoseries [GS] adhesin) to nonsecretor cells. The host-parasite interactions proposed to explain these observations is that the fucosyl transferase controlled by the secretor gene might modify molecules surrounding the receptors in the P antigen or the receptors themselves [20]. Since strains with the MR:non-GS fimbriae also were bound in greater numbers to the nonsecretor cells, we suggest an additional mechanism: that the Lewis a antigen found on nonsecretor cells is one of the receptors for adhesins on the surface of *E. coli*. Our studies indicate that Lewis a antigen is one of the receptors for *Candida* species [21]. We also found that pretreatment of the yeast with boiled saliva from nonsecretors significantly enhanced the binding of the yeast to epithelial cells [7, 8]. In 1982 Epstein and co-workers reported that preincubation of blastospores with a proportion of saliva samples from which specific antibodies to *Candida* had been adsorbed enhanced their binding to epithelial cells [22]. Saliva from ~20%–25% of their donors had this property, the proportion of nonsecretors predicted for an outbred population.

The nature of the Lewis antigens suggests the following model. Unlike the ABO antigens, the Lewis antigens do not form part of the structure of the cell. They are adsorbed from body fluids onto the surfaces of cells. If there are adhesins on *E. coli* that recognize Lewis antigens, the following interactions might occur: (*1*) the bacteria could bind directly to the epithelial cell via Lewis antigens or (*2*) the car-

Table 2. Three hypotheses proposed to explain the host-parasite interactions underlying increased susceptibility of nonsecretors to infections.

(1)	The carbohydrate moiety of glycoproteins present in the body fluids of secretors can bind to lectin-like adhesins on microorganisms, decreasing their ability to adhere to and colonize a mucosal surface.
(2)	The Lewis a antigen found in significant quantities only on the cells of nonsecretors is a receptor for some microbial adhesins.
(3)	The reported lower levels of serum and secretory IgA found in nonsecretors leads to a compromised state at their mucosal surfaces.

bohydrate moiety of free Lewis antigens in body fluids might bind to the adhesin and then be adsorbed onto the cell surface. We have evidence for this model with *Candida albicans* and are presently investigating it with *E. coli* isolates.

Hypothesis 3: The deficient immune response hypothesis. The third hypothesis is based on reports of lower levels of serum and secretory IgA in nonsecretors [23, 24]. If nonsecretors have lower levels of these antibodies that are classically associated with protection of the mucosal surfaces, their host defenses against UTI might be compromised. In comparison with age-matched controls, young girls with recurrent UTI have been found to have lower levels of total secretory IgA [25].

In the present paper we report our epidemiologic survey of 718 women with recurrent UTI and discuss our results in the context of the secretor states reported for 77 young Swedish girls with pyelonephritis followed by the research groups in Göteborg. We also present the data from our experiments in which we tested our third hypothesis – that nonsecretors are unable to produce an effective IgA response.

Patients and Methods

The population examined consisted of 718 women referred to the Pyelonephritis Clinic, City Hospital, Edinburgh, for specialist investigation of recurrent UTI. The women in both the patient and control groups are mainly of Celtic and Norse backgrounds; there is not a substantial immigrant population in this area. This population was divided into a prospective group of 516 women referred to the clinic during or after 1982 when the study began and a retrospective study group of 202 women followed by the clinic for 20 years. The control groups was 334 blood donors 18–65 years old [11].

Two hundred women in the retrospective study were divided into the following groups according to information in their clinical records (for a detailed account of this group see [26]): 54 women with renal scars as determined by pathological examination after nephrectomy (19 patients) or radiologic evidence (35 patients) and 146 with a history of recurrent UTI but no radiologic evidence of scarring. Voiding cystograms were not performed for all patients since these are considered invasive procedures not justifiable for routine examinations. The mean age of the women with renal scars was 50.5 years (range, 23–74 years) and that of the women with no evidence of scars was 54 years (range, 27–84 years). The difference in ages was not statistically significant [26].

Patients were considered to have improved if they had fewer documented episodes of infection during the second decade as compared with the first decade and not to have improved if they had more episodes of infection during the second decade than in the first decade. There were also a group of women for whom there was no change in the numbers of infections during the two decades. Among the group with renal scars, the mean number of infections for 42 patients during the first decade was five (range, 1–20) and was 2.5 (range, 0–10) for the second decade. For the group with no evidence of renal scarring, the mean number of documented infections for 96 patients during the first decade was 4.6 (range, 0–23) and was 2.8 (range, 0–35) for the second decade [26].

Secretor state was determined by the hemagglutination-inhibition assay described by Mollison [27], and the total serum IgA and IgG levels were determined with Behring Nor-partigen immunodiffusion plates. Total IgA and IgG levels were determined because from our epidemiologic studies there appeared to be no single strain or antigen associated with organisms causing recurrent UTI among these women [28]. The values for IgA and IgG were recorded in international units (IU).

The results for the women in the retrospective group were compared with those obtained for 100 age-matched women from the prospective study group recently referred to the clinic. In the analysis of these results with reference to secretor state, improvement, and renal scarring, we used data obtained for women 23–64 years old since the age range for which the Nor-partigen plates were standardized is 16–64 years [29].

The χ^2 test was used to compare the incidence of nonsecretors in the study with the incidence we reported for local blood donors (26.6%) [11]. Student's *t* test was used to compare IgA and IgG values for the different patient categories.

Results

The secretor states for the patients in the various categories are shown in table 3. A significantly higher proportion of women referred to the clinic for investigation of recurrent UTI were nonsecretors as compared with the proportion among the local control population. This increase was seen in the group with

Table 3. Proportion of nonsecretors among women referred for investigation of recurrent urinary tract infection.

Category (no.)	No. (%) nonsecretors	*P*
Total (718)	250 (34.8)	<.01
Prospective group (516)	172 (33.3)	<.05
Retrospective group (202)	78 (38.6)	<.01
Without renal scars (146)	53 (36.6)	<.05
With renal scars (54)	23 (42.6)	<.05
Improved (127)	47 (37)	<.05
Not improved (50)	20 (40)	NS
No change (22)	10 (45.5)	NS
Controls (334)	89 (26.6)	

NOTE. NS = not significant.

renal scarring, those with no evidence of scarring, and those who had improved over the 20 years. There were 29 women with juvenile onset of symptoms, 51% were nonsecretors ($P < .05$).

Table 4 compares the total serum IgA and IgG levels for the different patient categories. In contrast to our prediction, the mean IgA level for nonsecretors was higher than that for secretors as was the mean IgG level. The increased levels of IgA for nonsecretors were found for the group as a whole, for patients with renal scarring, for those with no evidence of scarring, and for those who had improved over the 20 years. This difference between the IgA levels of secretors and nonsecretors was not observed among the patients who had not improved or among those for whom there was no change in the incidence of infections over the two decades.

The differences were not due to the age of the population. Comparison of these figures with those obtained for the age-matched group of women recently referred to the clinic indicated that these differences between secretors and nonsecretors were found only in the group of women who had had these infections over a long period. Both IgA and IgG values for women in the retrospective study were higher than those for the age-matched women in the prospective study (table 5).

The immunoglobulin levels were analyzed with reference to the presence or absence of renal scarring. Among the women with no evidence of renal scarring, the only statistically significant difference found was the increased IgA levels for nonsecretors who had improved as compared with the levels for secretors who had improved. Although the mean for IgA was higher for the nonsecretors who had improved than for those who had not improved, this difference was just outside statistical significance (table 6).

The pattern observed among the women with scarred kidneys was more complex (table 7). Both IgA and IgG levels for the improved nonsecretors were significantly higher than those for the improved

Table 4. Geometric means of total serum IgA and IgG levels from secretors and nonsecretors in the different diagnostic categories.

	Geometric mean immunoglobulin level (IU/mL)			
Category (no.)	IgA	*P*	IgG	*P*
Total patients				
Secretor (111)	103	<.01	118	<.0025
Nonsecretor (70)	125.4		139.7	
Without renal scars				
Secretor (82)	107.9	<.05	123.3	NS
Nonsecretor (49)	127.4		138.7	
With renal scars				
Secretor (29)	90.3	<.0025	104.3	<.025
Nonsecretor (19)	121.8		151	
Improved				
Secretor (74)	99.3	<.0025	117.2	<.01
Nonsecretor (44)	133.1		140.7	
Not improved				
Secretor (24)	115.1	NS	125.4	NS
Nonsecretor (16)	113.3		133.8	
No change				
Secretor (11)	108	NS	114.5	NS
Nonsecretor (9)	112		153.1	

NOTE. NS = not significant.

Table 5. Geometric means of total serum IgA and IgG levels from secretors and nonsecretors in the retrospective and prospective studies.

	Geometric mean of immunoglobulin level (IU/mL)			
Patient category (no.)	IgA	*P*	IgG	*P*
Secretors				
Retrospective (111)	103	<.01	118	NS
Prospective (65)	82.2		108	
Nonsecretors				
Retrospective (71)	127.1	<.001	146	<.001
Prospective (35)	89.7		104	

NOTE. NS = not significant.

Table 6. Geometric means of total serum IgA and IgG levels from secretors and nonsecretors without renal scars with reference to improvement over 20 years.

	Geometric mean immunoglobulin level (IU/mL)			
Category (no.)	IgA	*P*	IgG	*P*
Improved				
Secretor (50)	107.8	<.05	121.3	NS
Nonsecretor (31)	136		138	
Not improved				
Secretor (19)	111.6	NS	137.3	NS
Nonsecretor (13)	106		130.7	
No change				
Secretor (11)	107.9	NS	114.5	NS
Nonsecretor (5)	136		168.3	
Mean value (healthy Central Europeans 15–64 years old)	125		144	

NOTE. NS = not significant.

secretors. The IgG levels of secretors who had not improved are intriguing. Although the numbers are small, four of the five patients in this category had unusually low levels of IgG as compared with the mean reported for central Europeans: 144 IU/mL [29].

Discussion

The results of this study refute our hypothesis that recurrent UTI among nonsecretors is due to their inability to mount an effective immune response. The results indicate that the increase in the proportion of nonsecretors among patients with recurrent UTI is not associated with an inability to produce IgA antibodies. Nonsecretors appear to be more dependent on their specific humoral responses than are secretors, perhaps because the innate protection associated with the secretor gene is absent.

A similar pattern was found in a separate study in which the total immunoglobulin levels in serum and saliva of carriers of *Neisseria meningitidis* were compared with those of non-carrier controls matched for age, sex, and secretor state. We were unable to confirm the reported lower levels of salivary IgA in nonsecretors in either the carrier or control groups; however, the levels of IgA in the saliva of carriers of both secretors and nonsecretors were double the levels in the controls [30]. Because the colonization/carriage of mucosal pathogens was not assessed, it is difficult to comment on the report of lower levels of IgA in the vaginal secretions of young girls with UTI as compared with those of age-matched girls with no history of UTI [25]. There was a significant increase in levels of total serum IgA in the nonsecretor carriers as compared with those of the nonsecretor controls. These higher levels of IgA were elicited by the capsulate, serogroupable strains but not by the nonserogroupable, nonpathogenic strains [30].

The presence or absence of serum antibodies specific for certain pathogens appears to influence their carriage on the host's mucosal surfaces. A reduction in the carriage of serogroup C *N. meningitidis* followed the immunization of recruits with group C polysaccharide vaccine [31], and the probability of becoming a carrier of serogroup B meningococci was greater in recruits who lacked detectable antibodies to serogroup B at induction [32]. *Candida albicans* was isolated more frequently from women with low levels of specific anti-*Candida* serum IgA but not from those with low levels of specific anti-*Candida* IgG [33]. Gough and co-workers found a significant correlation between anti-*Candida* IgA in serum and secretions [34]. If a similar response is elicited by infecting *E. coli* strains, the increased levels of serum antibodies might reflect increased levels of these at the mucosal surfaces.

Determination of the antigens against which the raised levels of IgA are directed might provide clues to those that induce protection. Regardless of age of onset of UTI or of the therapy prescribed, the majority of the patients had improved over the 20

Table 7. Geometric means of total serum IgA and IgG levels of secretors and nonsecretors with renal scars with reference to improvement over 20 years.

	Geometric mean immunoglobulin level (IU/mL)			
Category (no.)	IgA	*P*	IgG	*P*
Improved				
Secretor (24)	83.9	<.01	109.1	<.01
Nonsecretor (12)	128.5		156.9	
Not improved				
Secretor (5)	128.9	NS	84.1	<.05
Nonsecretor (3)	149.5		149.4	
Mean value (healthy Central Europeans 15–64 years old)	125		144	

NOTE. NS = not significant.

years, and improvement was associated with higher levels of IgA. The patient from whom the most isolates (12) were obtained during the study is a secretor, but her IgA levels were low (85.4 IU/mL) and her IgG levels average (144 IU/mL).

The ability to secrete the ABO blood group antigens appears to play a role in the host's innate defenses against a number of infectious agents, particularly those that affect nonimmune children [9, 10] and adults who are immunocompromised in some way [7, 8]. The proportion of nonsecretors is significantly increased among patients with invasive infections due to pneumococci, meningococci, or type b *Haemophilus* [9, 10]. These are diseases that are prevalent in children during the vulnerable period (6 months to 5 years) when maternal antibodies have declined and the child's own immune responses are not well developed [35]. Most UTI that result in kidney scars occur during this time [36]. There was a significant increase in the proportion of nonsecretors among our patients with juvenile onset of symptoms (51%) and among those with renal scars (42.6%). In a study of 77 Swedish children, there was a significant increase in the proportion of nonsecretors (40%) among those who developed kidney scars following UTI [37].

These observations suggest the following interactions. In the nonimmune secretor, the secreted glycoproteins might limit the numbers of bacteria at the attachment/colonization step of the pathogenic process. These small numbers of bacteria might elicit a modest inflammatory response and also induce specific antibodies. In the nonimmune nonsecretor, colonization might not be inhibited but might be actively enhanced via the interactions with Lewis a antigen, as suggested by our in vitro studies of *Candida* and by the increased attachment of uropathogenic *E. coli* to nonsecretor cells [20]. The larger numbers of colonizing bacteria might multiply in the absence of specific immune protection and induce a strong inflammatory response. If this takes place in the kidney, scarring might result.

The recent report of Johnson et al. [38] suggests that the specific immune responses of the host might also contribute to the pathogenesis of scarring. Binding of IgA-containing immune complexes by tissue macrophages in the lung elicits production of reactive oxygen radicals, resulting in significant damage to surrounding tissues. We have suggested that if the immune responses in children parallel those observed in adults, the higher levels of IgA and IgG found in nonsecretors might contribute to pathogenesis of renal scarring by similar mechanisms [39]. There are reports of local production of IgA in the urinary tract and significant levels of both IgA and IgG in the urine of children with pyelonephritis [40]. If complement is present, IgG-containing complexes also can elicit tissue damage [41].

The pathogenesis of UTI is multifactorial. The finding that nonsecretion of the ABO antigens is associated with recurrent infections provides a stable host marker for investigation of interactions between the nonspecific defenses and the immune responses to the infecting organism, both in the initiation of infection and possible pathogenic sequelae.

References

1. Mourant AE. ABH secretion and natural selection. Ann Hum Biol 1982;9:575–7
2. Mourant AE. Blood relations: blood groups and anthropology. Oxford: Oxford University Press, 1983:125
3. Thordarson G, Bjarnason O, Lincoln PJ, Dodd BE. ABH secretor and Lewis type frequencies in an Icelandic series. J Med Genet 1972;9:46–7
4. Chown B, Lewis M. The blood groups and the secretor genes of the Stoney and Sarcee Indians of Alberta, Canada. Am J Phys Anthropol NS 1955;13:181–90
5. Chown B, Lewis M. The inheritance of the blood group and secretor genes in the Blood Indians of Alberta, Canada. Am J Phys Anthropol NS 1955;13:473–8
6. Chaudhuri A, DasAdhikary CR. Possible role of blood secretory substances in the aetiology of cholera. Trans Roy Soc Trop Med Hyg 1978;72:664–5
7. Blackwell CC, Thom SM, Lawrie OR, Weir DM, Wray D, Kinane DF. Non-secretion of blood group antigens and susceptibility to oral infections by *Candida albicans* [abstract no. 135]. J Dent Res 1986;65:502
8. Blackwell CC, Thom SM, Weir DM, Kinane DF, Johnstone FD. Host-parasite interactions underlying non-secretion of blood group antigens and susceptibility to infections by *Candida albicans*. In: Lark D, ed. Protein carbohydrate interactions in biological systems. London: Academic Press, 1986:231–3
9. Blackwell CC, Jonsdottir K, Hanson M, Todd WTA, Chaudhuri AKR, Mathew B, Brettle RP, Weir DM. Non-secretion of ABO antigens predisposing to infection by *Neisseria meningitidis* and *Streptococcus pneumoniae* [letter]. Lancet 1986;2:284–5
10. Blackwell CC, Jonsdottir K, Hanson MF, Weir DM. Non-secretion of ABO blood group antigens predisposing to infection by *Haemophilus influenzae* [letter]. Lancet 1986;2:687
11. Kinane EF, Blackwell CC, Brettle RP, Weir DM, Winstanley FP, Elton RA. ABO blood group, secretor state and susceptibility to recurrent urinary tract infection in women. Br Med J 1982;285: 7–9
12. Blackwell CC, May SJ, Brettle RP, Maccallum CJ, Weir DM.

Host-parasite interactions underlying non-secretion of blood group antigens and susceptibility to recurrent urinary tract infections. In: Lark D, ed. Protein carbohydrate in interactions in biological systems. London: Academic Press, 1986:229–30

13. May SJ, Blackwell CC, Brettle RP, Maccallum CJ, Weir DM. Non-secretion of ABO blood group antigens: a factor predisposing to recurrent urinary tract infections and renal scarring (submitted for publication)
14. Shinebaum R, Blackwell CC, Forster PJG, Hurst NP, Weir DM, Nuki G. Non-secretion of ABO blood group antigens as a host susceptibility in the spondyloarthropathies. Br Med J 1987;294:208–10
15. Blackwell CC, James VS, Weir DM, Gemmill JD, Patrick AW, Collier A, Clarke BF. Secretor state of patients with insulin dependent or non-insulin dependent diabetes mellitus. Br Med J 1988;95:1024–5
16. Oriol R, Le Pendu J, Mollicone R. Genetics of ABO, H, Lewis, X and related antigens. Vox Sang 1986;51:161–71
17. Williams RC, Gibbons RJ. Inhibition of streptococcal attachment to receptors on human buccal epithelial cells by antigenically similar salivary glycoproteins. Infect Immun 1975;11:711–8
18. Holmgren J, Svennerholm A-M, Åhrén C. Nonimmunoglobulin fraction of human milk inhibits bacterial adhesion (hemagglutination) and enterotoxin binding of *Escherichia coli* and *Vibrio cholerae*. Infect Immun 1981;3:136–41
19. Andersson B, Porras O, Hanson LÅ, Lagergård T, Svanborg Edén C. Inhibition of attachment of *Streptococcus pneumoniae* and *Haemophilus influenzae* by human milk and receptor oligosaccharides. J Infect Dis 1986;153:232–7
20. Lomberg H, Cedergren B, Leffler H, Nilsson B, Carlström A-S, Svanborg Edén C. Influence of blood group on the availability of receptors for attachment of uropathogenic *Escherichia coli*. Infect Immun 1986;51:919–26
21. May SJ, Blackwell CC, Weir DM. Non-secretion of blood group antigens and susceptibility to *Candida albicans*: the role of Lewis blood group antigens [abstract no. 136]. J Dent Res 1986;65:503
22. Epstein JB, Kimura LH, Menard TW, Truelove EL, Pearsall NN. Effects of specific antibodies on the interaction between the fungus *Candida albicans* and human oral mucosa. Arch Oral Biol 1982;27:469–74
23. Grundbacher FJ. Immunoglobulins, secretor status, and the incidence of rheumatic fever and rheumatic heart disease. Hum Hered 1972;11:399–404
24. Waissbluth JG, Langman MJS. ABO blood groups, secretor status, salivary protein, and serum and salivary immunoglobulin concentrations. Gut 1971;12:646–9
25. Tuttle JP Jr, Sarvas H, Koistinen J. The role of vaginal immunoglobulin A in girls with recurrent urinary infections. J Urol 1978;120:742–4
26. Brettle RP, MacCallum CJ, Murdoch J, Gray JA. The adult female – a 20 year study of bacteriuria in female patients. In: Asscher AW, Brumfitt W, eds. Microbial diseases in nephrology. New York: John Wiley and Sons, 1986:257–71
27. Mollison PL. Blood transfusion in clinical medicine. 6th ed. Oxford: Blackwell Scientific Publications, 1979:414–82
28. May SJ, Rahat A, Blackwell CC, MacCallum CJ, Brettle RP, Weir DM. Characterization of *Escherichia coli* strains isolated from urine of secretors and non-secretors (submitted for publication)
29. Becker W, Rapp W, Schwick HG, Störiko K. Methoden zur quantitativen Bestimmung von Plasmaproteinen durch Immunopräzipitation. Zeitschrift für Klinische Chemie und Klinische Biochemie 1968;6:113–22
30. Blackwell CC, Weir DM, James VS, Cartwright KAV, Stuart J, Jones DM. Non-secretion of ABO blood group antigens and susceptibility to infectious diseases. In: NATO advanced workshop: complement, phagocytosis and bacteria. Maratea, Italy, 1987
31. Gotschlich EC, Goldschneider I, Artenstein MS. Human immunity to the meningococcus. V. The effect of immunization with meningococcal group C polysaccharide on the carrier state. J Exp Med 1969;129:1385–95
32. Kalapothaki V, Vassiliadis P, Trichopoulos D, Kaklamani E, Sérié C. Charactéristiques séro-(epidémiologiques chez les porteurs sains de méningocoques en Grèce. Ann Microbiol (Inst Pasteur) 1979;130B:43–54
33. Schonheyder H, Johansen JA, Møller-Hansen C, Stenderup A. IgA and IgG serum antibodies to *Candida albicans* in women of child-bearing age. Sabouraudia 1983;21:223–31
34. Gough PM, Warnock DW, Richardson MD, Mansell NJ, King JM. IgA and IgG antibodies to *Candida albicans* in the genital tract secretions of women with or without vaginal candidosis. Sabouraudia 1984;22:265–71
35. Greenwood BM. Selective primary health care: strategies for control of disease in the developing world. XIII. Acute bacterial meningitis. Rev Infect Dis 1984;6:374–89
36. Rolleston GL, Maling TMJ, Hodson CJ. Intrarenal reflux and the scarred kidney. Arch Dis Child 1974;49:531–9
37. Lomberg H, Hellstrom M, Jodal U, Svanborg Edén C. Secretor state and renal scarring in girls with recurrent pyelonephritis (submitted for publication)
38. Johnson KJ, Ward PA, Kunkel RG, Wilson BS. Mediation of IgA induced lung injury in the rat. Role of macrophages and reactive oxygen products. Lab Invest 1986;54:499–506
39. Blackwell CC, May SJ, Brettle RP, MacCallum CJ, Weir DM. Secretor state and immunoglobulin levels among women with recurrent urinary tract infections. J Clin Lab Immunol 1987;22:133–7
40. Åkerlund AS, Ahlstedt S, Hanson LÅ, Jodal U. Antibody responses in urine and serum against *Escherichia coli* O antigen in childhood urinary tract infection. Acta Pathol Microbiol Scand [C] 1979;87:29–36
41. Johnson KJ, Wilson BS, Till GO, Ward PA. Acute lung injury in rat caused by immunoglobulin A immune complexes. J Clin Invest 1984;74:358–69

Epidemiol. Infect. (1990), **104**, 203–209
Printed in Great Britain

Secretor status, smoking and carriage of *Neisseria meningitidis*

C. C. BLACKWELL[1], D. M. WEIR[1], V. S. JAMES[1], W. T. A. TODD[2], N. BANATVALA[2], A. K. R. CHAUDHURI[2], H. G. GRAY[3], E. J. THOMSON[4] AND R. J. FALLON[5]

[1] *Department of Bacteriology, University of Edinburgh, Edinburgh*
[2] *Infectious Diseases Unit, Monklands District General Hospital, Airdrie*
[3] *Department of Community Medicine, Monklands Cumbernauld District, Lanarkshire Health Board, Airdrie*
[4] *Microbiology Laboratory, Monklands District General Hospital, Airdrie*
[5] *Meningococcus (Scotland) Reference Laboratory, Ruchill Hospital, Glasgow*

(*Accepted 20 November 1989*)

SUMMARY

A survey of ABO blood groups, secretor status and smoking habits among 389 students and staff of a school in which there was an outbreak of meningococcal disease found no difference in the distribution of the ABO blood groups but a significantly higher proportion of non-secretors (37·6%) in the population examined compared with that reported for previous surveys of the neighbouring population in Glasgow (26·2%) ($P < 0{\cdot}0005$). There was also a significantly higher proportion of non-secretors among carriers of meningococci (47%) compared with non-carriers (32%). Increased carriage of meningococci among non-secretors might contribute to the increased susceptibility of individuals with this genetic characteristic to meningococcal disease observed in previous studies. Although passive exposure to cigarette smoke has been associated with meningococcal disease, there was no association between passive smoking and carriage. There was, however, a significant association between active smoking and carriage.

INTRODUCTION

In December 1988 there was an outbreak of disease due to a serogroup B serotype 4 P1.15 sulphonamide-resistant strain of *Neisseria meningitidis* in Airdrie, Lanarkshire. There were five cases of the disease among students at the Airdrie Academy; one teacher developed the disease and died. Four of the cases occurred in the second year, three of these in one form. A child who attended another school was also infected.

During the week before the Christmas/New Year recess, teams from the Infectious Diseases Unit at Monklands District General Hospital and the Department of Community Medicine screened 1492 students and staff for carriage of meningococci, and issued each with a course of rifampicin (600 mg bd for 2 days). During the second week of January 1989 when the school had reopened, a selected group of students and staff was retested for carriage of meningococci and

specimens of blood and saliva were also obtained. In addition, participants were interviewed about smoking habits or passive exposure to cigarette smoke that has been associated with meningococcal infections.

The genetically determined inability of an individual to secrete the glycoprotein form of his ABO blood group antigens (non-secretion) is associated with susceptibility to meningococcal infection [1]. Prolonged outbreaks of meningococcal disease have occurred in areas where there are higher proportions of non-secretors [2, 3]. In this study we determined if there is also a higher proportion of non-secretors in a population in which there was a single short outbreak of the disease. In the study in Stonehouse, Gloucestershire we found an increase in the proportion of non-secretors among carriers of meningococci within the community, but this was not significantly different from the proportion of non-secretors from whom no meningococci or *Neisseria lactamica* were isolated. In this study we determined if there is a higher proportion of non-secretors among carriers of meningococci within the school population.

Several environmental factors have been associated with susceptibility to meningococcal disease: passive exposure to cigarette smoke [4, 5]; overcrowded accommodation [6]; poverty and lower social class [7]; strenuous physical activity [5]; predisposing viral infections [8–10]. As the population affected was old enough to have enough smokers for statistical analysis, the effects of smoking or passive exposure to cigarette smoke on carriage of meningococci among students and staff was examined.

SUBJECTS AND METHODS

In December 1988, as part of an urgent public health measure to administer prophylactic antibiotics, 1492 staff and students at the Airdrie Academy were screened for carriage of meningococci by nasopharyngeal swabbing. In January 1989, 389 selected individuals at the school were screened for carriage of meningococci. From the second year class, 201 of the 226 pupils were examined as this was the year in which 4 of the 5 cases among the children occurred. From the other five classes, 85 carriers plus a group of 85 non-carriers from the same form matched as closely as possible for age and sex were examined: year one, 25 students; year three, 39 students; year four, 68 students; year five, 29 students; year six, 9 students. From the staff, there were ten members who were carriers matched as closely as possible for age and sex with eight non-carriers with similar duties in the school. Each participant supplied a saliva sample (5 ml), and 10 ml of blood was obtained from 371.

ABO and Lewis blood groups were determined by agglutination of blood specimens with monoclonal antibodies. Because the haemagglutination inhibition test for secretion with saliva can yield 'false' secretors if contaminated with blood, the Lewis test was used to determine secretor status: secretors express Lewis^b and non-secretors express Lewis^a. The nasopharyngeal swabs were cultured on GC selective medium (NYC/Thayer Martin medium) and *N. meningitidis* identified by Gram stain, oxidase test and the Gonochek II system (EY Laboratories). The serogroup, serotype and sensitivities to sulphonamide and rifampicin were determined at Ruchill Hospital, Glasgow.

Each participant in the January survey was interviewed to obtain information

regarding smoking habits and exposure to cigarette smoke. The information was coded and analyzed with the laboratory results by a Database II programme. Because all the participants could not be matched for sex, the results were compared by the χ^2 test rather than McNemar's test.

RESULTS

Among the 1492 students and staff tested in December 1988 *N. meningitidis* was isolated from 126 (8·4%). There were 11 isolates which were serogroup B or non-groupable with increased resistance to sulphonamide and with the serotype and/or subtype of the outbreak strain; 6 of these were isolated from students in the form (2A) where 3 of the 5 cases occurred. In December none of the isolates was resistant to rifampicin; however, in January 3 of the 13 isolates, including 2 with characteristics of the outbreak strain, were resistant to rifampicin.

ABO blood group distribution and secretor status of the school population

Blood specimens were obtained from 371 students and staff. The distribution of the ABO groups is shown in Table 1; this does not appear to differ markedly from that observed in earlier studies for either Edinburgh [11] or Glasgow [12]. The distribution of the Lewis^a/non-secretors and the Lewis^b/secretors in the population tested does differ significantly from the figures reported earlier for Edinburgh [11] ($\chi^2 = 9{\cdot}03$, $P < 0{\cdot}005$) and Glasgow [13] ($\chi^2 = 12{\cdot}14$, $P < 0{\cdot}0005$) (Table 2). Among the 185 students in the second year class whose red cells expressed Lewis antigen, 65 (35%) were Lewis^a/non-secretors, a significantly higher proportion compared with the figure for nearby Glasgow ($\chi^2 = 4{\cdot}81$, $P < 0{\cdot}05$). In form 2A where 3 of the 5 cases among the students occurred, 13 of the 26 (50%) children tested were non-secretors; but this was not significantly increased compared with the other forms in year 2. Only 2 of the 5 cases were non-secretors.

Specimens were obtained from 109 individuals who had been carriers in December. There were 51 non-secretors (47%) among the carriers compared with 83 non-secretors among the 262 non-carriers (32%) ($\chi^2 = 4{\cdot}94$, $P < 0{\cdot}05$). The majority of these strains did not have the characteristics of the outbreak strain. Specimens were obtained from 10 of the 11 students who were carriers of isolates with characteristics of the outbreak strain; 6 of the 10 carriers were non-secretors.

Meningococci were isolated from 13 of the 389 tested in January and 5 of the carriers were non-secretors (38·5%). The outbreak strain was isolated from 2 students, one secretor and one non-secretor, both of whom had received rifampicin.

Smoking and carriage of meningococci

There was no association between passive exposure to cigarette smoke (father and/or mother smokers) and carriage; but there was an association between students' smoking habits and carriage. There were 17 carriers among the 37 students who were smokers (46%) compared with 87 carriers among 349 students who were non-smokers (25%) ($\chi^2 = 6{\cdot}48$, $P < 0{\cdot}025$) (Table 3). Four (31%) of the 13 students who were carriers in January were smokers. In the December survey, 6 of the 10 members of staff who were carriers were smokers, but none of the eight

Table 1. *Comparison of the distribution of ABO blood groups for the Airdrie Academy population with those of Glasgow and Edinburgh*

	Blood group							
	A		B		O		AB	
	No.	(%)	No.	(%)	No.	(%)	No.	(%)
Airdrie	118	(32)	47	(13)	198	(53)	8	(2)
Glasgow	1906	(32)	637	(11)	3177	(54)	178	(3)
Edinburgh	104	(31)	42	(13)	173	(52)	15	(4)

Table 2. *Comparison of secretor status of the Airdrie population with those of Glasgow and Edinburgh*

	Lewis^b/secretor		Lewis^a/non-secretor			
	No.	(%)	No.	(%)	χ^2	P
Airdrie	222	(62·4)	134	(37·6)	—	—
Glasgow	371	(73·8)	132	(26·2)	12·14	<0·0005
Edinburgh	245	(73·4)	89	(26·6)	9·03	<0·005

Table 3. *Carriage of meningococci among smokers and non-smokers*

	Carrier		Non-carrier	
	No.	(%)	No.	(%)
Smoker (37)	17	(46)	20	(54)
Non-smokers (349)	87	(25)	262	(75)
Total	104		282	

non-carrier controls from the staff were smokers. The proportion of girls who smoked 27/208 (13%) was significantly higher than that of boys who smoked 10/171 (6%) ($\chi^2 = 4{\cdot}64$, $P < 0{\cdot}05$). The proportion of carriers was, however, similar for both boys and girls, 47/171 (28%) and 57/208 (27%) respectively.

DISCUSSION

The proportion of non-secretors in most western European populations is 20–25% [14]. In areas where there have been prolonged outbreaks of meningococcal disease such as Iceland, Nigeria and Stonehouse in Gloucestershire, we have found increased proportions of non-secretors [2, 3]. The present study provided an opportunity to determine if the same pattern would be found in short localized outbreaks. Although the distribution of the ABO blood groups was similar to that previously reported for Glasgow and Edinburgh (Table 1), the proportion of Lewis^a non-secretors was 37·6% among the staff and students tested, significantly higher than those previously reported for Glasgow (26·2%) [13] or Edinburgh (26·6%) [11] (Table 2). This increase in the proportion of Lewis^a/non-secretors is similar to that observed among the residents of Stonehouse, Gloucestershire (32·7%) compared with blood donors in the south west region (23·4%) [3].

In the survey reported here, there was a significant increase in the proportion of non-secretors who were carriers of meningococci (47%) compared with that among the non-carriers (32%). In the Stonehouse study, the proportion of non-secretors among carriers did not differ significantly from that of the participants from whom no neisseria were isolated [3].

The main reason for the difference in the two studies is probably an underestimate of the proportion of non-secretors among the carriers in the Stonehouse survey [15]. The secretor status of each of the participants in the Stonehouse survey was determined from the saliva specimen collected. Blood contamination of saliva specimens can result in false 'secretors', so the Lewis blood group was determined for a proportion of the blood specimens as a control for secretor status. Although the ABO blood group was determined for each specimen at the time of collection, the large number of individuals examined in the Stonehouse survey (>6000) limited the determination of the Lewis blood group to every eighth specimen.

For the specimens obtained in the Stonehouse study, the results of the saliva and the Lewis blood group agreed for more than 92% of the pairs of specimens tested. In the report on the Stonehouse data, it was emphasized that agreement between the two tests was closest in the areas of the town where socioeconomic conditions were better [3]. Differences between the blood and saliva tests indicating 'false' secretors occurred significantly among the specimens obtained from residents of Park Estate and Verney Fields where (*a*) 14 of the 15 cases of meningococcal disease occurred [15], (*b*) the carrier rates for both outbreak and non-outbreak strains were higher [16] and (*c*) the socioeconomic indicators were lower [15]. Blood in saliva might be due to poor oral hygiene, periodontal disease or smoking; each can affect the integrity of the oral mucosa and each is associated with poorer socioeconomic conditions.

The results of this study provide evidence for our hypothesis that one of the factors contributing to the susceptibility of non-secretors to meningococcal disease is increased carriage of these bacteria among such individuals. This parallels the reports of increased proportions of non-secretors among patients with rheumatic fever and among carriers of *Streptococcus pyogenes* [17]. A significant increase in the proportion of non-secretors has also been found among healthy carriers of *Candida albicans* [18], and we have found a similar pattern among patients with non-insulin dependent diabetes mellitus [19].

Passive smoking is associated with meningococcal disease [4, 5], but we found no association with passive exposure to cigarette smoke (mother and/or father who were smokers) and carriage of meningococci. There was however, a significant increase in the proportion of carriers among smokers (46%) compared with that among non-smokers (25%) ($P < 0{\cdot}025$). The higher proportion of girls who were smokers (13%) compared with boys who were smokers (6%) agrees with the recently reported trends of increased smoking among teenage girls [20].

A slightly higher proportion of males to females has been noted among carriers of meningococci; in the study of Cartwright and co-workers [16] the ratio was 3:2, males:females. If smoking has a significant effect on carriage, in the population examined in the present study, the higher proportion of girls who smoked might contribute to the similar proportions of boys (28%) and girls (27%) who were

carriers. Analysis of smoking and secretor status among carriers and non-carriers indicate that these are two independent factors affecting carriage.

ACKNOWLEDGEMENTS

We wish to thank the staff and students of Airdrie Academy for their cooperation and help, the Edinburgh and South East Scotland Blood Transfusion Service for reagents used in the study. Dr K. A. V. Cartwright, Dr J. M. Stuart and Mrs P. Robinson for helpful discussions and Mrs M. K. Cole for preparation of the manuscript. The project was supported by grants from Scottish Hospitals Endowment Research Trust, The Meningitis Trust, TENOVUS-Scotland, the Medical Research Council and the Lanarkshire Health Board.

REFERENCES

1. Blackwell CC, Jonsdottir K, Hanson M, et al. Non-secretion of ABO antigens predisposing to infection by *Neisseria meningitidis* and *Streptococcus pneumoniae*. Lancet 1986; ii: 284–5.
2. Blackwell CC, Jonsdottir K, Mohammed I, Weir DM. Non-secretion of blood group antigens – a genetic factor predisposing to infection by *Neisseria meningitidis*. In Poolman JT ed., et al., Gonococci and meningococci. Dordrecht: Kluwer Academic Publishers, 1988.
3. Blackwell CC, Weir DM, James VS, Cartwright KAV, Stuart JM, Jones DM. The Stonehouse study: secretor status and carriage of *Neisseria* species. Epidemiol Infect 1989; **102**: 1–10.
4. Stuart JM, Cartwright KAV, Dawson AJ, Rickard J, Noah ND. Risk factors for meningococcal disease: a case control study in Southwest England. Community Med 1988; **10**: 139–46.
5. Haneberg B, Tonjum T, Rodahl K, Gedde-Dahl T. Factors preceding the onset of meningococcal disease with special emphasis on passive smoking, stressful events, physical fitness and general symptoms of ill health. National Inst Public Health Annals 1983; **6**: 169–74.
6. Kaiser AB, Hennekens CH, Saslaw MS, Hayes PS, Bennett JV. Sero-epidemiology and chemoprophylaxis of disease due to sulphonamide resistant *Neisseria meningitidis* in a civilian population. J Infect. Dis 1974; **130**: 217–24.
7. Farries JS, Dickson W, Greenwood E, Malhotra TR, Abbot D, Jones DM. Meningococcal infection in Bolton 1971–1975. Lancet 1975; ii: 18–21.
8. Nichol KP, Cherry JD. Bacterial-viral interrelations in respiratory infections of children. N Engl J Med 1967; **277**: 667–72.
9. Eichenwald K, Kotsevalov O, Fasso LA. Some effects of viral infection on ariel dissemination of staphylococci and on susceptibility to bacterial colonization. Bacteriol Rev 1961; **25**: 274–81.
10. Krakinski K, Nilson JD, Butler S, Laby JP, Kusmiesz H. Possible association of mycoplasma and viral respiratory infections with bacterial meningitis. J Epidemiol 1987; **125**: 499–508.
11. Kinane DF, Blackwell CC, Brettle RP, Weir DM, Winstanley FP, Elton RA. Blood group secretor state and susceptibility to recurrent urinary tract infections in women. Br Med J 1982; **285**: 7–9.
12. MacSween MP, Syme UA. ABO blood groups and skin diseases. Brit J Dermatol 1965; **77**: 30–4.
13. Cameron JM. Blood groups in tumors of salivary tissue. Lancet 1958; i: 239–40.
14. Mourant AE, Kopec A, Domaniewska-Sobszak K. The distribution of the human blood groups and other polymorphisms, 2nd ed. London: Oxford University Press, 1976.
15. Stuart JM, Cartwright KAV, Jones DM, et al. An outbreak of meningococcal disease in Stonehouse: planning and execution of a large-scale survey. Epidemiol Infect 1987; **99**: 579–89.
16. Cartwright KAV, Stuart JM, Jones DM, Noah ND. The Stonehouse survey, nasopharyngeal carriage of meningococci and *Neisseria lactamica*. Epidemiol Infect 1987; **99**: 591–601.

17. Haverkorn MJ, Goslings WRO. Streptococci, ABO blood groups and secretor status. Amer J Human Genet 1969; **21**: 360–75.
18. Burford-Mason AP, Weber JCP, Willoughby JMT. Oral carriage of *Candida albicans*, ABO blood group and secretor status in healthy individuals. J Med Vet Mycol 1988; **26**: 49–56.
19. Blackwell CC, Aly FZM, Weir DM, et al. Blood group, secretor status and oral carriage of yeasts among patients with diabetes mellitus. Diabetes Res. In press.
20. Wald N, Kiryluk S, Darby S, Doll R, Pike M, Peto R. UK smoking statistics. Oxford: Oxford University Press, 1988.

rejection episode needing treatment during the first postoperative month (controls *vs* UDCA treated group, $p < 0.01$, Fisher's exact test). Patients have been followed for a median of 5 months (range 3–8) and controls for 12 months (range 3–15 months). Aminotransferases were significantly lower in the UDCA-treated patients than in controls 1 month after transplantation (table).

The lack of acute rejection in the UDCA treated liver transplant recipients cannot be explained by improved surgical technique since the liver transplant programme and the surgeons in our unit have not changed since 1985. In addition, the immunosuppression protocol remained the same throughout 1989.

We used UDCA to achieve a less toxic bile acid pool which would protect the hepatocytes and prevent cholestasis.[1-5] UDCA has a direct protective effect on hepatocytes in both in-vivo and in-vitro studies.[6] Calmus and coworkers[7] have shown that UDCA reduces class I HLA-antigen expression on hepatocytes in patients with primary biliary cirrhosis. HLA class I antigens are not usually expressed on the hepatocytes,[8] but this effect of UDCA indicates that it may also have other immunoeffects and the potential to alter antigen expression in cells other than hepatocytes in such patients.

Department of Surgery,
Sahlgrenska Hospital,
University of Göteborg,
S-413 45 Göteborg, Sweden

HANS PERSSON
STYRBJÖRN FRIMAN
TORE SCHERSTÉN
JOAR SVANVIK
INGVAR KARLBERG

1. Poupon R, Chretien Y, Poupon RE, Ballet F, Calmus Y, Darnis F. Is ursodeoxycholic acid an effective treatment for primary biliary cirrhosis? *Lancet* 1987; i: 834–36.
2. Ullrich D, Rating D, Schröter W, Hanefeld F, Bircher J. Treatment with ursodeoxycholic acid renders children with biliary atresia suitable for liver transplantation. *Lancet* 1987; ii: 1324.
3. Ghezzi C, Zuin M, Bartezzati PM, Podda M. Effects of ursodeoxycholate, taurine and ursodeoxycholate plus taurine on serum enzyme levels in patients with chronic hepatitis. *Hepatology* 1987; 7: 1110.
4. Nimura H, Tohira A, Hayashi M, et al. Ursodeoxycholic acid therapy in the treatment of biliary atresia. *Biomed Pharmacother* 1989; 43: 37–41.
5. O'Brien C, Senior JR, Batta AK, et al. Ursodeoxycholic acid treatment produces marked clinical and biochemical amelioration of primary sclerosing cholangitis. *Gastroenterology* 1989; 96: 640.
6. Heuman DM, Komito SF, Pandak WM, Hylemon PB, Vlahcevic ZR. Tauroursodeoxycholic acid protects against cholestatic and hepatocytolytic toxicity of more hydrophobic bile salts. *Gastroenterology* 1989; 96: A607.
7. Calmus Y, Gane P, Rouger P, Poupon R. Hepatic expression of class I and class II major histocompatibility complex molecules in primary biliary cirrhosis: effect of ursodeoxycholic acid. *Hepatology* 1990; 11: 12–15.
8. Rouger PH, Poupon R, Gane P, Mallissen B, Darnis F, Salomon CH. Expression of blood group antigens including HLA markers in human adult liver. *Tissue Antigens* 1986; 27: 78–86.

Meningococcal disease: high virulence and low transmission

SIR,—Dr Knight and his colleagues (May 19, p 1182) suggest that the prolonged outbreak of meningococcal disease in the Gloucester area and in Plymouth might be due to local environmental factors, a genetically susceptible population, or a more virulent strain of the organism. Their evidence indicates that isolates from patients in these two areas are from a single clone, a sulphonamide-resistant, serogroup B, serotype 15, serosubtype P1.16 strain of *Neisseria meningitidis* (B15/16 R). They suggest that this strain has remained localised in these regions because it is of increased virulence but poorly transmissible.

Individuals who are genetically incapable of secreting the water-soluble form of their ABO blood group antigens (non-secretors) are significantly over-represented among patients with meningococcal disease[1] and among carriers of meningococci.[2] We showed a significant increase in the proportion of non-secretors in Stonehouse, the Gloucestershire town where there has been an unusually high attack rate of disease due to the B15/16R strain,[3,4] and in a Scottish school population in which there was an outbreak due to a serogroup B, serotype 4, serosubtype P1.15, sulphonamide-resistant strain (B4/15R).[2]

100 individuals from the Plymouth area were examined for their Lewis blood group antigen as controls for a study of the Lewis phenotype among patients with breast cancer.[5] Non-secretors can express only Lewis^a and secretors express Lewis^b. If the controls are representative of the Plymouth population, the proportion of Lewis^a non-secretors is significantly higher (37·6%) than that of blood donors in the south west region (chi-squared test $p < 0.0005$; table).

PREVALENCE OF NON-SECRETORS IN THREE BRITISH POPULATIONS IN WHICH OUTBREAKS OF MENINGOCOCCAL DISEASE OCCURRED

Location	Outbreak strain	Total Le^a/Le^b	Le^a/non-secretor (no [%])	p value
South west				
Blood donors	..	632	148 (23·4)	..
Stonehouse	B15,16R	411	134 (32·6)	<0·005
Plymouth	B15/16R	91	34 (37·4)	<0·0005
Scotland				
Glasgow	..	503	132 (26·2)	..
Airdrie Academy	B4,15R	356	134 (37·6)	<0·0005

In addition to the hypothetical increased virulence and poor transmissibility of the B15/16R clone, we suggest that the higher proportion of the genetically susceptible non-secretors in the two areas might contribute to the localisation of these outbreaks.

Department of Bacteriology,
Medical School,
University of Edinburgh,
Edinburgh EH8 9AG, UK

C. C. BLACKWELL
D. M. WEIR

1. Blackwell CC, Jonsdottir K, Hanson M, et al. Non-secretion of ABO antigens predisposing to infection by *Neisseria meningitidis* and *Streptococcus pneumoniae*. *Lancet* 1986; ii: 284–85.
2. Blackwell CC, Weir DM, James VS, et al. Secretor status, smoking and carriage of *Neisseria meningitidis*. *Epidemiol Infect* 1990; 104: 203–09.
3. Blackwell CC, Weir DM, James VS, Cartwright KAV, Stuart JM, Jones DM. The Stonehouse study: secretor status and carriage of *Neisseria* species. *Epidemiol Infect* 1989; 102: 1–10.
4. Stuart JM, Cartwright KAV, Jones DM, et al. An outbreak of meningococcal disease in Stonehouse: planning and execution of a large scale survey. *Epidemiol Infect* 1987; 99: 579–89.
5. Phippa RF, Perry PM. Lewis-negative genotypes and breast cancer. *Lancet* 1989; i: 1198–99.

DNA probes for typing *Neisseria meningitidis*

SIR,—We were most interested in the use of a DNA probe to investigate genetic heterogeneity among *Neisseria meningitidis* B15P1.16 strains, described by Dr Knight and colleagues (May 19, p 1182). They used a probe that detects a small (2 kb) repeated sequence and we are not certain that the results obtained using this probe are epidemiologically valid. The B15P1.16 sulphonamide-resistant strains that are prevalent in the UK and elsewhere have been shown to be clonally related by isoenzyme electrophoresis but it is reasonable to expect that there are degrees of genetic heterogeneity within this clone that will be large enough to code for variations in surface components and possibly virulence factors. We have been using a randomly selected probe prepared from a genomic library of B15P1.16 prepared in λWesλB phage to type meningococci by restriction fragment length polymorphisms (RFLPs). This probe appears to react with all meningococci that we have tested and has a high degree of discrimination and reproducibility. When we applied this probe to B15P1.16 strains collected over the past decade from many parts of the UK we obtained results that differ from those of Knight et al and lead to a fundamentally different interpretation. In short, we find that there are two main variants of the B15P1.16 strains scattered about the UK and that the Plymouth strains have a different RFLP pattern to the Gloucester strains. The difference is demonstrable in isolates from 1982 and has remained stable and detectable through to the 1990 isolates.

We have also had the opportunity to use a monoclonal antibody (prepared by Dr J. E. Heckels, Southampton) that detects a point mutation in the class I outer membrane protein P1.16 on the B15P1.16 isolates. With this entirely different approach the

Autoimmunity, 1990, Vol. 7, pp. 279–289
Reprints available directly from the publisher
Photocopying permitted by license only

SECRETOR STATUS AND INFECTION IN PATIENTS WITH GRAVES' DISEASE

A.D. TOFT[+], C.C. BLACKWELL[*++], A.T. SAADI[++], P. WU[+], P. LYMBERI[+++], M. SOUDJIDELLI[+++] and D.M. WEIR[++]

Departments of Medicine[+] and Bacteriology[++], The Medical School, University of Edinburgh, Edinburgh, Scotland, and Hellenic Institute Pasteur[+++], Athens, Greece

We have demonstrated that the inability to secrete the water soluble glycoprotein form of the ABO blood group antigens into saliva is significantly more common in patients with Graves' disease than control subjects (40% vs 27%; $P < 0.025$) but not among those with Hashimoto's thyroiditis or spontaneous primary atrophic hypothyroidism. Non-secretion is associated with increased susceptibility to infection and to asymptomatic carriage of some microorganisms.

Although *Yersinia enterocolitica* has been found to express antigen cross reactive with the TSH receptor, we did not find an increased prevalence of *Yersinia* species in the faeces of 107 patients with Graves' disease. The isolation rate (< 1%) was similar to that observed in the local population with diarrhoeal illness. Salivary IgA levels determined by whole cell ELISA with *Y. enterocolitica* 03 were not elevated in the majority of specimens examined. The results suggest that, in contrast to reports from Scandinavia, there is no strong evidence that yersiniae play a role in the pathogenesis of Graves' disease among patients in South east Scotland.

Non-secretors are significantly over represented among patients with several other autoimmune diseases; however, with the exception of antitubulin antibodies, non-secretors with Graves' disease did not have more antibodies to other human antigens than secretor patients.

KEY WORDS: Graves' disease, non-secretors, *Yersinia enterocolitica*, Salivary IgG.

INTRODUCTION

Although there is considerable debate about the host-parasite interactions involved, there is a growing body of evidence that infectious agents play a role in triggering several autoimmune diseases. One of the hypotheses put forward to support this proposal is that of molecular mimicry: there are antigens present on micro-organisms that are cross-reactive with those of human tissues. As these cross-reacting antigens are widespread among different species of microorganisms, some additional genetic or environmental factors have been proposed to be involved in the aetiology of these autoimmune diseases.

Two different sets of genetic factors have been identified. Compared with unaffected individuals, the distributions of the HLA antigens among patients with certain autoimmune diseases suggest that affected individuals differ in their immune responses [Table 1]. Our research group has found a second genetic factor outside the HLA system to be over-represented among patients with several autoimmune diseases for which infectious aetiologies have been suggested. This is non-secretion, the

*Corresponding author.

Table 1 HLA antigens associated with autoimmune diseases for which infectious triggers have been proposed

Disease	*HLA marker(s)*	
ankylosing spondylitis	B27	[1]
reactive arthritis	B27	[2, 3]
palmoplantar pustulosis	B8, CW7, DR3	[5]
insulin dependent diabetes	B8, B15, DR3, DR4	[6]
Graves' disease	B8, DR3	[7]

inability of an individual to secrete the water-soluble form of his ABO blood group antigens into body fluids such as saliva or urine. The carbohydrate moieties of the ABO blood group antigens and the closely related Lewis blood group antigens exist in two forms. The glycolipid form is found as part of the structure of cell membranes; the glycoprotein form is found in the body fluids and secretions. As the secretor gene (*Se*) is inherited in a dominant pattern, approximately 75–80% of most European populations are secretors while 20–25% express the recessive non-secretor phenotype. The secretor gene also influences expression of the Lewis blood group antigens; non-secretors produce only Lewis^a and secretors express small amounts of Lewis^a but mainly Lewis^b. This provides a check on secretor status which is usually determined by a haemagglutination inhibition assay. The ABO and Lewis blood group antigens found on cells and in body fluids of secretors and non-secretors are compared in Table 2

Non-secretors are significantly over-represented among patients with infections due to a variety of bacteria and fungi [Table 3] (for review see[8,9]); and non-secretors are also over-represented among asymptomatic carriers of *Streptococcus pyogenes*, *Neisseria meningitidis* and *Candida albicans*[10–12].

Investigation of secretor status of patients with autoimmune diseases for which infectious aetiologies have been proposed suggest that non-secretion plays a part in susceptibility to a variety of these conditions [Table 3]. Antigens or microoriganisms that cross-react with host tissues have been suggested to play a role in triggering several diseases [Table 5]; and, in three of these conditions, an association with non-secretion has been identified: rheumatic fever, seronegative arthropathies and Graves' disease.

The evidence for an infectious trigger, particularly *Yersinia*, in Graves' disease is summarized in Table 6. Although there is not usually clinical evidence or history of diarrhoeal disease preceeding this condition, we examined the following hypotheses.

1. If *Yersinia* is one of the triggers for Graves' disease, we might find cultural or

Table 2 Blood group antigens in secretors and non-secretors

BG antigen	*Secretors*		*Non-secretors*	
	On cells	*In secretions*	*On cells*	*In secretions*
H (A/B)	+	+	+	−
Lewis^a	− (+)*	− (+)*	+	+
Lewis^b	+	+	−	−

*Present in small quantities

Table 3 Infectious agents to which non-secretors are more susceptible

GRAM-POSITIVE	–	*Streptococcus pyogenes* *Streptococcus penumoniae*
GRAM-NEGATIVE	–	*Escherichia coli (UTI)* *Neisseria meningitidis* *Haemophilus influenzae* *Vibrio cholerae*
YEASTS	–	*Candida albicans*

[for review see 8, 9].

serological evidence of carriage of these bacteria among patients on their referral to the endocrinology clinic

2. As non-secretors are over-represented among patients with several autoimmune diseases, there is not a specific bacterial species involved in the aetiology of Graves' disease; but, non-secretors are more likely to produce autoantibodies to a variety of self antigens.

METHODS

Patients

One hundred and seven patients with Graves' disease were studied who had been referred consecutively as new patients to the same physician (ADT) at the Endocrine Clinic, Royal Infirmary, Edinburgh, were included in the study. There were 89 females and 18 males with a mean age of 46.2 ± SE 8.1 years. The diagnosis of Graves' disease was based on the demonstration of raised serum concentrations of free thyroxine and/or total triiodothyronine and undetectable thyrotrophin (TSH) measured by radioimmunoassays[29,30]. At least one of the following was also present: diffuse goitre with an overlying bruit; exophthalmos; pretibial myxoedema; and a concentration of TSH-receptor antibodies in the serum in excess of 5 units per litre[31].

Table 4 Autoimmune diseases and non-secretion

Disease	*Association with non-secretion*	
Rheumatic diseases		
rheumatic fever	yes	[10]
ankylosing spondylitis	yes	[13]
reactive arthritis	yes	[13]
palmoplantar pustulosis	yes	[14]
rheumatoid arthritis	no	[14]
psoriasis	no	[15]
psoriatic arthropathy	yes	[15]
Endocrine diseases		
diabetes (undifferentiated)	yes	[16]
insulin dependent diabetes	yes	[17]
non-insulin dependent diabetes	no	[17]
Graves' disease	yes	[18]
Hashimoto's thyroiditis	no	[18]
primary atrophic hypothyroidism	no	[18]

Table 5 Bacteria with antigens crossreactive with host tissues

Disease	*Microorganism*	*antigen*
rheumatic fever	*Streptococcus pyogenes*	myocardium [19]
seronegative arthropathies	*Shigella flexneri* *Yersinia enterocolitica* *Klebsiella pneumoniae*	HLA B27 [20]
myasthenia gravis	*Yersinia enterocolitica*	acetylcholine receptor [21]
Graves' disease	*Y. enterocolitica* Gram positive and gram negative commensals	TSH receptor [22, 23, 24]

ABO and Lewis blood groups of patients were determined by agglutination with monoclonal antibodies provided by the Edinburgh and South East Scotland Blood Transfusion Service.

Isolation and identification of Yersinia *spp. from stool specimens*

Each stool specimen was inoculated onto agar selective for yersiniae, cefsulodin-irgasan-novobiocin (C.I.N.) After 24 h at 28°C, the colonies with a typical dark red "bull's eye" surrounded by a transparent border were subcultured onto blood agar and incubated at 28°C overnight. Gram-stain, oxidase and urease tests were done with colonies from the blood agar plate. Urease positive, oxidase negative colonies were examined with API 20E strips.

As cold enrichment results in a selective growth of yersiniae at the expense of other enterobacteria, the stool samples were incubated at 4°C for 3 weeks and were cultured again on C.I.N. media.

ELISA for total IgA in saliva

Saliva specimens from patients with Graves' disease were examined by enzyme linked immunosorbent assay (ELISA). A pool of 50 saliva specimens obtained from individuals with no endocrine disease was used as a standard for the study.

Rabbit anti-human IgA (Behringer) was diluted in coating buffer (50 mM sodium carbonate buffer, pH 9.6) and 100 μl of a 1/500 dilution were added to individual wells

Table 6 Evidence for infectious triggers in autoimmune thyroid disease

1. Antibodies to *Yersinia* are often found in sera of patients with thyroid disease[25-27]
2. Saturable binding sites for TSH have been demonstrated on *Yersinia* and commensal Gram-negative and gram-positive bacteria[22-24]
3. Affinity studies suggest bacterial proteins were the immunogen for antibodies reactive with the TSH receptor[24]
4. The rat model autoimmune thyroid disease suggests normal flora of the animals play a role in triggering the disease[28]

of Immulon IV M129 D plates (Dynatech). The plates were incubated at 4°C overnight then washed 3 times with washing buffer, 0.01 M phosphate buffer saline (PBS) with 0.1% bovine serum albumin (BSA) and 0.05% Tween 20. Blocking buffer PBS with 1% BSA (100 μl) was added to each well, and, after 30 mins incubation at room temperature, the plates were washed 3 times.

Saliva specimens diluted 1/1000 in PBS (100 μl) were added to the plates and incubated for 2 hrs at room temperature. The plates were washed 3 times and 100 μl of a 1/100 dilution of rabbit anti-human IgA (α-chain) conjugated with horseradish peroxidase (HRP) (DAKO) were added to the wells and incubated for 2 hrs. The plates were washed 3 times and 50 μl of the substrate, 40 mg of ortho-phenylenedamine (OPD) in 100 ml of 0.1 phosphate citrate buffer (pH 5) activated with 40 μl of 30% H_2O_2, were added to the wells and incubated for 20 mins in the dark at room temperature. The reaction was stopped by adding 50 μl of 12.5% sulfuric acid to each well. The plates were read at 490 nm optical density using a Dynatech MR700 Microplate reader. All specimens were tested in duplicate.

Detection of salivary IgA specific for Yersinia enterocolitica *03 and Escherichia coli*

Y enterocolitica 03 (YO3), the most common yersinia serotype observed in European populations, obtained from Dr. M.F. Hanson, Central Microbiological Laboratory, Edinburgh was maintained at room temperature on egg storage slopes. An *E. coli* strain was isolated from a stool specimen on MacConkey agar and stored on egg storage slopes. YO3 was grown on CIN medium at 28°C for 24 hrs and *E. coli* was grown on MacConkey's medium at 37°C for 24 hrs. Bacteria were suspended in 2% formal saline for 30 min, after which heavy suspensions were prepared in coating buffer and kept at 4°C. The numbers of bacteria per ml were standardized by optical densities at 540 nm; an optical density of 1.00 was used for each of the organisms.

Bacteria in coating buffer (100 μl) were added to wells of the ELISA plates and incubated overnight at 4°C. All other procedures were carried out at room temperature. The plates were washed 3 times with washing buffer and 100 μl of blocking buffer was added to each well. After incubation for 30 minutes the blocking buffer was removed, the plates washed 3 times and 100 μl of a 1/10 dilution (PBS) of the saliva specimens were added to the wells and incubated for 2 hrs at room temperature. From this point the procedure used for determination of total salivary IgA was carried out.

All specimens were tested in duplicate.

Saliva specimens with high OD readings in the screening assay for antibodies to YO3 were mixed with an equal volume of a heavy suspension of YO3 or an equal volume of saline and the suspensions were incubated overnight at 4°C. The tubes were centrifuged at 3,000 × g and the supernatants tested in the ELISA for presence of antibody to YO3.

Measurement of autoantibodies

Sera from 66 secretors (38 from the present study and 28 from a previous study) and 47 non-secretors (20 from the present study and 27 from a previous study) were examined by ELISA for the presence of IgA and IgG antibodies against a panel of autoantigens: thyroglobulin, insulin, single-stranded DNA (SS DNA) double-stranded DNA (DS DNA), actin, tubulin, myosin, keratin and collagen type I and the hapten trinitrophenol (TNP)[32,33]. Igm rheumatoid factors were detected by the

Waaler-Rose test and antibodies to microsomal antigens by a passive haemagglutination technique. The results were expressed as the percentage absorbance (optical density) of the test serum compared with the reference pool (defined as 100%). Specimens with adsorbance values 3 standard deviations (3SD) (usually > 300%) above the mean value for normal levels were considered to be positive.

RESULTS

Secretor status and autoimmune thyroid disease

In our previous study, patients with autoimmune thyroid diseases – Graves' disease, Hashimoto's thyroiditis and primary atrophic hypothyroidism (PAH) were examined for the distribution of their ABO blood groups and secretor status. There was no unusual distribution of the ABO blood groups among the three patient groups. There was, however, a significant increase in the proportion of non-secretors among those with Graves' disease but not among those with Hashimoto's thyroiditis or PAH [Table 7].

Isolation of yersiniae

Fresh stool specimens from 107 patients with Graves' disease were cultured by standard methods described above. The specimens were cultured a second time after incubation at 4°C for 3 weeks to enrich for yersiniae. Only one isolate was obtained, a *Y. enterocolitica* serotype 03, an isolation rate of < 1%.

Salivary antibodies to yersiniae

There was a very wide range of ELISA values in the assay for YO3 and for the strain of *E. coli* examined. Not all the low ELISA readings for YO3 were associated with low levels of total salivary IgA [Figure 1]. Adsorption of saliva with high values for YO3 with the test strain resulted in a marked reduction in the levels of IgA detected in the assay [Table 8]. The antibodies were not totally specific for YO3 as adsorption with *E. coli* and *Neisseria meningitidis* also partly reduced the levels of antibody detected, suggesting some of the response might be due to cross-reactivity among Gram-negative bacteria.

Table 7 Secretor status of patients with autoimmune thyroid diseases

	Secretors		*non-secretors*		*P*
	n	(%)	*n*	(%)	
Graves' disease (n = 100)	60	(60)	40	(40)	< 0.025
Hashimoto's thyroiditis (n = 30)	21	(70)	9	(30)	NS
PAH (n = 35)	25	(71)	10	(29)	NS
Controls (n = 334)	245	(73)	89	(27)	

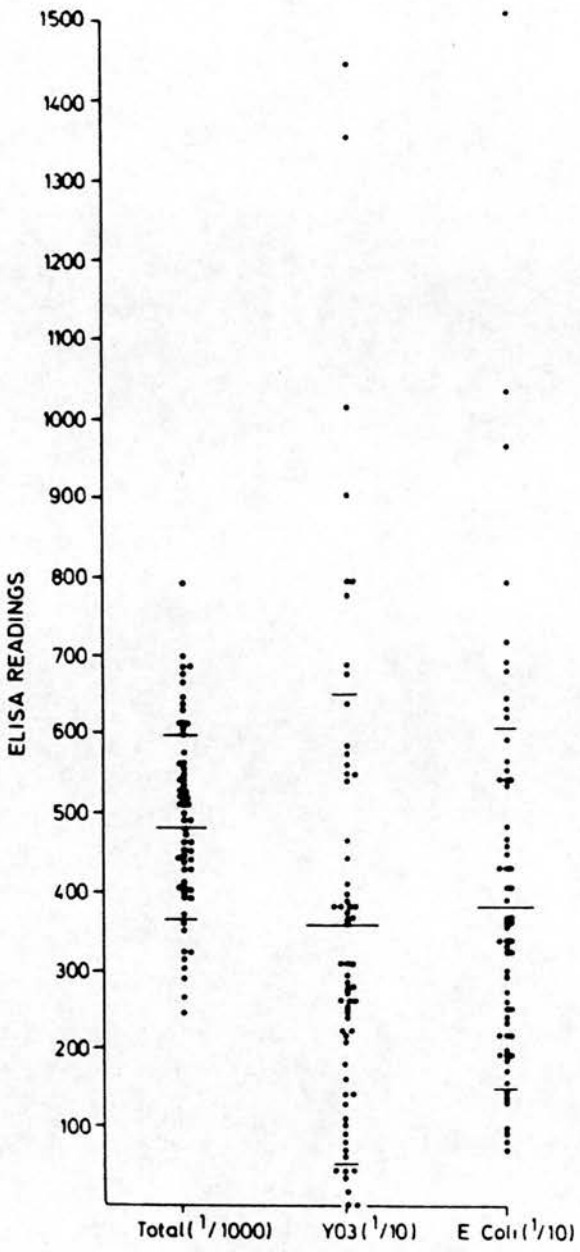

ELISA READINGS
1500
1400
1300
1200
1100
1000
900
800
700
600
500
400
300
200
100
Total(1/1000)
Y03(1/10)
E Coli(1/10)

Figure 1

Table 8 Salivary IgA levels in whole cell ELISA for *Y enterocolitica* 03 before and after adsorption with the test strain

Patient number	*ELISA reading unadsorbed*	*absorbed*
57	.803	−0.040
69	.436	−0.005
92	>2.000	1.023

Autonatibodies in patients with Graves' disease

None of the sera from patients with Graves' disease contained rheumatoid factors. The proportions of secretors and non-secretors with levels of antibodies to microsomal antigens > 1/100 were 41% and 36% respectively.

The results for the ELISA against the various antigens are shown in Table 9 and 10. None of the patients sera had antibodies to collagen. There was no significant difference between the proportions of secretors and non-secretors with IgA antibodies to any of the antigens examined [Table 9]. There appear to be more non-secretors with IgA antibodies to thyroglobulin than secretors, but larger numbers of patients need to be screened to confirm this trend. The only significant difference in IgG auto-antibodies of secretors and non-secretors was that the sera of a higher proportion of non-secretors contained anti-tubulin autoantibodies [Table 10].

DISCUSSION

Our studies suggest that although yersiniae might be the bacteria expressing antigens cross-reactive with the TSH receptor in some populations, it is not obviously the source of these antigens in the Scottish patients examined. We isolated only one strain from stool specimens from 107 patients with Graves' disease. An isolation rate of < 1% is similar to that observed among patients in the Edinburgh area examined for diarrhoeal disease [Dr. M.F. Hanson, personal communication]. *Yersinia* are not

Table 9 IgA autoantibodies in patients with Graves' disease

ANTIGEN	*% Patients with autoantibodies** *secretor n = 66*	*non-secretor n = 47*
THYROGLOBULIN	0	8.5+
INSULIN	1.5	2
DS DNA	0	0
SS DNA	6	8.5
ACTIN	1.5	0
TUBULIN	9.1	4.2
MYOSIN	3	2
KERATIN	6	4.2
COLLAGEN I	0	0
TNP	6	2

+ 0.05 > p < 0.1 (not statistically significant)

*Elisa reading > 3 SD above normal range

Table 10 IgG autoantibodies in patients with Graves' disease

ANTIGEN	*% Patients with autoantibodies**	
	secretor $n = 66$	*non-secretor* $n = 47$
THYROGLOBULIN	53	47
INSULIN	13.6	10.6
DS DNA	1.5	0
SS DNA	7.6	2.1
ACTIN	3	0
TUBULIN	1.5	12.8**
MYOSIN	12.6	10.6
KERATIN	3	6.4
COLLAGEN I	0	0
TNP	7.6	17

**$P < 0.05$
*Elisa reading > 3 SD above normal range

common pathogens in Great Britain and the carriage rate among patients with Graves' disease is not increased. Byfield and colleagues found antigens cross-reactive with the TSH receptor to be present on *E. coli* and other species that form the normal flora of the bowel[24]. Studies are underway to examine faecal flora of patients with Graves' disease for presence of bacteria with these antigens.

The results for salivary IgA specific for yersiniae were not conclusive. Although a number of specimens appeared to have high levels of IgA for YO3 [Figure 1] [Table 8], adsorption of the saliva specimens with *E. coli* and *N. meningitidis* suggest the probability of cross-reactions with other Gram-negative flora cannot be excluded. Screening of sera from these patients for antibodies to the plasmid coded proteins described by Wenzel and colleagues[27] might provide more specific serological evidence.

Although non-secretors are significantly over-represented among patients in studies of a variety of autoimmune diseases, we have found no evidence for our second hypothesis: non-secretors have significant amounts of autoantibodies to a greater number of self antigens than secretors. None of the patients with Graves' disease had autoantibodies to rheumatoid factor or type I collagen. Similar proportions of secretors and non-secretors had significant levels of IgA or IgG antibodies to insulin, TNP, SS DNA, DS DNA actin, myosin, and keratin. The only significant difference between secretors and non-secretors was the higher proportion of non-secretors with IgG antibodies to tubulin, an autoantibody reported to be increased among patients with autoimmune thyroid disease[34].

We have found an association between non-secretion and some organ specific autoimmune diseases (Graves' disease and insulin dependent diabetes mellitus). Our studies of non-organ specific autoimmune conditions such as rheumatoid arthritis and psoriasis have not found this pattern. If infectious agents act as triggers for the organ-specific diseases, the higher proportion of non-secretors with these conditions might reflect their initial susceptibility to bacteria that express the cross-reactive antigens. Colonization with these bacteria might not result in clinical symptoms of infection but might precipitate the sequence of immunological interactions resulting in production of autoantibodies associated with these diseases.

References

1. Brewerton DA, Caffney M, Hart ED, James DCO, Nicholls A, Sturrock RD. Ankylosing spondylitis and HL-A27. *Lancet* 1973; **i:** 904–907
2. Aho K, Ahvonen R, Alkio P, Lassus A, Sairanen E, Sievers K, Tulikainen A. HL-A27 in reactive arthritis following infections. *Annal Rheum Dis* 1975; **34** (supplement): 29–30
3. Håkansson U, Löw B, Eitrem R, Winblad S. HL-A27 and reactive arthritis in an outbreak of salmonellosis. *Tissue Antigen* 1975; **6:** 366–67
4. Leirisalo M, Skylr G, Kousa M, Voipio-Pulkki L-M, Suoranata H, Nisslä M., Hvidman L, Damm Nielsen E, Svejgaard A, Tiilikainen A, Laitinen O. Follow up study on patients with Reiter's disease and reactive arthritis with special reference to HLA-B27. *Arthr Rheumat* 1982; **25:** 249–59
5. Kemmett D, Leigh IM, Baker HA, McCloskey D, Alonso A, Awad J, Festenstein H. Histocompatibility antigens in plaque psoriasis and palmo-plantar pustulosis. *Br J Dermatol* 1985; **113:** 40
6. Keen H. The genetics of diabetes: from nightmare to headache. *Br Med J* 1987; **294:** 917–19
7. Tiwari JG Terasaki PI. HLA and disease associations. New York, Springer Verlag, 1985
8. Blackwell CC. Genetic susceptibility to infectious agents. *Proc R Coll Phys of Edinburgh* 1985; **19:** 129–38
9. Blackwell CC. The role of ABO blood groups and secretor status in host defences. *FEMS Microbiol Immunol* 1989; **47:** 341–50
10. Haverkorn MJ, Goslings WRO. Streptococci, ABO blood groups and secretor status. *Am J Hum Genet* 1969; **21:** 360–375
11. Blackwell CC, Weir DM, James VS, Todd WTA, Banatvala N, Chaudhuri AKR, Gray HG, Thomson EJ, Fallon RJ. Secretor status, smoking and carriage of *Neisseria meningitidis*. *Epidemiol Infect* 1990; (in press)
12. Burford-Mason AP, Welsby JCP and Willoughby JMT. Oral carriage of *Candida albicans*, ABO blood group and secretor status in healthy individuals. *J Med Vet Mycol* 1988; **26:** 49–56
13. Shinebaum R, Blackwell CC, Forster PJG, Hurst NP, Weir DM, Nuki G. Non-secretion of ABO blood group antigens as a host susceptibility factor in the spondylartho-pathies. *Br Med J* 1987; **294:** 208–10
14. Sloan RL, Blackwell CC, Kemmett D, Weir DM, Nuki G. Non-secretion of ABO blood group antigens in patients with palmoplantar pustulosis: Evidence for an infective aetiology and a further distinction from psoriasis. [Submitted for publication].
15. Shinebaum R, Blackwell CC, Abernethy VE, Situnayake DR, Weir DM, Nuki G. Secretor state of patients with psoriasis, psoriatic arthropathy or rheumatoid arthritis. *Br J Rheumatol* 1987; **76** (suppl 2): 105
16. Andersen J, Lauritzen E. Blood groups and diabetes mellitus. *Diabetes* 1960; **9:** 20–24
17. Blackwell CC, James VS, Weir DM, Gemmell JD, Patrick AW, Collier A, Clarke BF. Secretor status of patients with insulin dependent or non-insulin dependent diabetes mellitus. *Br Med J* 1987; **295:** 1024–25
18. Collier A, Patrick AW, Toft AD, Blackwell CC, James VS, Weir DM. Increased prevalence of non-secretors in patients with Graves' disease – evidence for an infective aetiology? *Br Med J* 1988; **296:** 1162
19. Kaplan MH, Meyerserian M. An immunological cross-reaction between group A streptococcal cells and human heart tissue. *Lancet* 1962; **i:** 706–10
20. Van Bohemen CG, Grumet FC, Zanen HC. Identification of HLA-B27Ml and M2 cross-reactive antigens in *Klebsiella, Shigella* and *Yersinia*. *Immunol* 1984; **52:** 607–10
21. Stefansson K, Dieperink M, Richman DP, Marton LS. Sharing of epitopes by bacteria and the nicotinic acetylcholine receptor: a possible role in the pathogenesis of myasthenia gravis. *Ann NY Acad Sci* 1987; **506:** 461–69
22. Weiss M, Ingbar SH, Winblad S, Kaspar DL. Demonstration of a saturable binding site for thyrotropin in *Yersinia enterocolitica*. *Science* 1983; **219:** 1331
23. Heyma P, Harrison LC, Robins-Browne R. Thyrotropin (TSH) binding sites on *Yersinia enterocolitica* recognized by immunoglobulins from humans with Graves' disease. *Clin Exp Immunol* 1986; **64:** 249–54
24. Byfield PGH, Copping S, Davies SC, Barclay FE, Borriello SP. Interactions of TSH and Graves' IgG with bacterial binding proteins. *J Endocrinol* 1988; **117** (suppl 1): 283
25. Bech K, Larsen JH, Hansen JM, Nerup J. *Yersinia enterocolitica* infection and thyroid disease. *Lancet* 1974; **ii:** 951–952
26. Shenkman L and Bottone EJ. Antibodies against *Yersinia enterocolitica* in thyroid disease. *Annal Int Med* 1976; **85:** 735–739

27. Wenzel BE, Heesemann J, Wenzel KW, Scriba PC. Antibodies to plasmid-encoded proteins of enteropathogenic yersinia in patients with autoimmune thyroid disease. *Lancet* 1988; **i**: 56
28. Penhale WJ, Young PR. The influence of the normal microbial flora on the susceptibility of rats to autoimmune thyroiditis. *Clin Exp Immunol* 1988; **72**: 288–92
29. Challand GS, Ratcliffe WA, Ratcliffe JG. Semi-automated radioimmunoassays for thyroxine and triiodothyronine. *Clin Chem Acta* 1975; **60**: 25–32
30. Gow SM, Caldwell G, Toft AD, Beckett GJ. SimulTRAC™ simultaneous radioimmunoassay of thyrotropin and free thyroxine evaluated. *Clin Chem* 1986; **32**: 2191–94
31. Shewring G and Smith BR. An improved radioreceptor assay for TSH-receptor antibodies. *Clin Endocrinol* 1982; **17**: 409–17
32. Lymberi P, Konstandoulakis M, Boussiotis V, Pangalis G. Natural autoantibodies in sera of patients with B-chromic lymphocytic leukemia. Leukemia and lymphoma [submitted for publication].
33. Dighiero G, Guilbert B, Avrameas S. Naturally occurring antibodies against more common antigens in normal humans. I. Detection, isolation and characterisation. *J Immunol* 1982; **128**: 2779
34. Rousset B, Bernier-Valentin F, Poncet C, Orgiaazzi J, Madec A-M, Monier JC, Mornex R. Anti-tubulin antibodies in autoimmune thyroid disease. *clin Exp Immunol* 1983; **52**: 325–32

Vox Sang 613 FR gs

S. KARGER AG
Postfach, CH–4009 Basel

Semi-Quantitative Determination of H Type 1 and Type 2 Antigens on Buccal Epithelial Cells and in Saliva of Secretors and Non-Secretors

A. Rahat, J. Stewart, C. C. Blackwell, D. M. Weir

Department of Bacteriology, The Medical School, University of Edinburgh, UK

Key words. H Type 1 · H Type 2 · Secretor status · Buccal epithelial cells

Abstract. The amount of H antigens on buccal epithelial cells (BEC) of secretors and non-secretors was measured by flow cytometry. H type 1 and H type 2 on BEC were detected with fluorescein-labelled *Ulex europaeus* lectin, and H type 2 was measured with mouse monoclonal antibody. Between 3.5 and 6 times more H antigen was detected on cellls from secretors compared with cells from non-secretors. The level of H type 2 was the same on BEC of secretors and non-secretors. Adsorption of H type 1 antigen from secretor saliva onto non-secretors BEC was demonstrated. A simple and sensitive ELISA method was developed for the determination of H in saliva with biotin-labelled *Ulex* lectin. This system provides a simple means for measuring the amount of H present in saliva and for the determination of secretor status.

Introduction

The H blood group antigen found on various epithelial cells has been proposed to have a role in the susceptibility of non-secretors to bacterial infection [1]. It has been suggested that this molecule might be an attachment site for some micro-organisms. As a first step in the investigation of this hypothesis, we developed sensitive methods for measuring the two main types of H antigen found on BEC and in saliva.

H type 1 and H type 2 differ in their chemical composition, the genes that control their expression and their sites of production [2]. H type 1 is the main type of H antigen found in secretions. It is produced by fucosylation of type 1 precursor chains. The fucosyltransferase that synthesizes H type 1 is coded for by the secretor gene [3]. This antigen is not found in secretions of non-secretors, since they lack the secretor gene.

The expression of H type 2 antigen as part of the cell membrane in different tissues is independent of secretor status. The H gene codes for a fucosyltransferase that preferentially uses type 2 precursor chains [3]. Previous studies have shown that H type 1 and/or H type 2 antigens can be detected on the surface of buccal epithelial cells (BEC) and in saliva [4–7].

In the present study we measured the amount of H antigen on BEC in suspension by flow cytometry. By this method we were able: (1) to compare the expression of the two antigens in secretors and non-secretors and (2) to investigate the uptake of H antigen present in saliva onto BEC. The amount of H antigen in saliva (of secretors and non-secretors) was determined by a simple enzyme-linked immunosorbent assay (ELISA).

Materials and Methods

Subjects. The ABO blood groups of 34 donors were determined from heparinized blood samples by slide agglutination with monoclonal antibodies (Scottish National Blood Transfusion Service). Their secretor status was determined by the haemagglutination inhibition assay with saliva [8].

Collection of BEC. BEC from healthy individuals were collected by rubbing the inside of the cheek with cotton swabs. To remove the cells, swabs were rinsed in 20 ml Dulbecco's phosphate-buffered saline containing Ca^{2+} and Mg^{2+} ions, 0.9 and 0.4 m*M*, respectively (DPBS+B). The cells were washed twice in DPBS+B (300 *g* for 10 min) and their concentration adjusted to 2.5×10^5 cells/ml.

Collection of Saliva. Saliva was collected from healthy individuals and the samples were centrifuged at 1,000 *g* for 20 min. The supernatant was collected, boiled for 20 min, recentrifuged and stored at -20 °C until used.

Detection of H Antigen on BEC. The total amount of H (type 1 and type 2) was detected by fluorescein isothiocyanate (FITC) conjugated *Ulex europaeus* (UEAI), Sigma, Poole, Dorset, UK). H type 2 was measured using mouse monoclonal antibody (moAb) Im92/90, provided by Dr. R.H. Fraser, Glasgow, UK, and West of Scotland Blood Transfusion Service [9]. The antibody was purified on Synsorb H type 2 (Synsorb Chembiomed Ltd., Alberta, Canada) and conjugated with FITC by the method of Johnson and Holborow [10]. Two hundred microlitres of BEC were mixed with an equal volume of UEAI, moAb or buffer in a 5-ml culture tube and incubated at 37 °C for 15 min with continuous shaking. The cells were washed three times with DPBS+B (300 *g* for 10 min) and fixed with 1% buffered paraformaldehyde [11]. Analysis was done on an EPICS 'C' flow cytometer (Coulter Electronics, Luton, UK) equipped with a 5-watt laser using a power output of 300 mW at 488 nm. The BEC were selected from a display of forward angle light scatter versus 90° light scater by means of a bit map. More than 3,000 cells were analysed from each sample. The percentage of cells showing fluorescence greater than the background level was recorded on a one-parameter histogram, measuring fluorescence on a logarithmic scale. The mean fluorescence channel values for the positive cells were acquired from a one-parameter histogram, measuring fluorescence on a linear scale.

Adsorption of H Antigen from Saliva. BEC from secretors or non-secretors were mixed in 5-ml test tubes with equal volumes of undiluted secretor saliva from a blood group 0 donor. The tubes were shaken for 1 h at 37 °C and washed twice with DPBS+B (300 *g* for 10 min). The amount of H antigen on the cells was determined using UEAI and moAb as described above.

Detection of H Antigen in Saliva. The wells of polystyrene microtitres plates (M129B Dynatech, Billinghurst, Sussex, UK) were coated overnight at 4 °C with 100 µl of tenfold dilutions of saliva in 50 m*M* sodium carbonate buffer (pH 9,6). All further procedures were carried out at room temperature. The wells were washed three times with 0.01 *M* phosphate-buffered saline (PBS) containing 0.1% bovine serum albumin (BSA) + 0.05% Tween 20 (washing buffer). The wells were blocked with 150 µl of PBS containing 1% BSA (blocking buffer) for 15 min. The buffer was then removed and the wells were washed twice with washing buffer. One hundred microlitres of 5 µg/ml UEAI biotin labelled (Sigma, Poole, Dorset, UK), diluted in blocking buffer, were added to each well for 30 min. After 3 washes, 100 µl of 1/100 dilution of streptavidin biotinylated horseradish peroxidase complex (Amersham, Amersham, UK) in blocking buffer were added for 20 min. The wells were washed three times, and 100 µl of substrate solution containing 40 mg *O*-phenylenediamine and 40 µl H_2O_2 (30%) in 100 ml of 0.1 *M* phosphate citrate buffer (pH 5) were added. The reaction was allowed to develop in the dark for 20 min and stopped by adding 50 µl of 12.5% H_2SO_4. Absorbance at 490 nm was measured using a Dynatech plate reader (Dynatech, Billinghurst, Sussex, UK). Samples were run in duplicates and the readings averaged.

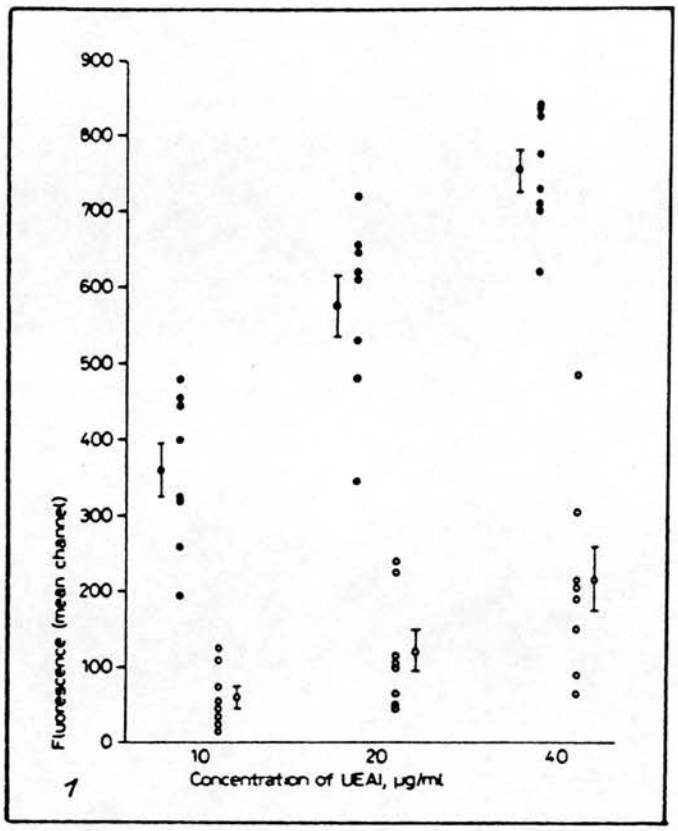


Fig. 1. Flow cytometric determination of H on BEC from secretors (●) and non-secretors (○). Bars represent mean ± 1 SEM.

Statistics. Student's t test was used for all statistical analysis.

Results

Figure 1 shows the levels of H antigen detected with UEAI on BEC of secretors and non-secretors by flow cytometry. The total amount of H antigen on BEC was between 3.5 and 6 times greater for secretors than for non-secretors, depending on UEAI concentration. This difference was statistically significant for the three concentrations of UEAI used in this study ($p<0.001$). Almost all the cells (90–99%) in the two populations expressed the H antigen; but a greater amount of antigen, as determined by mean channel, was present on secretor cells (fig. 2).

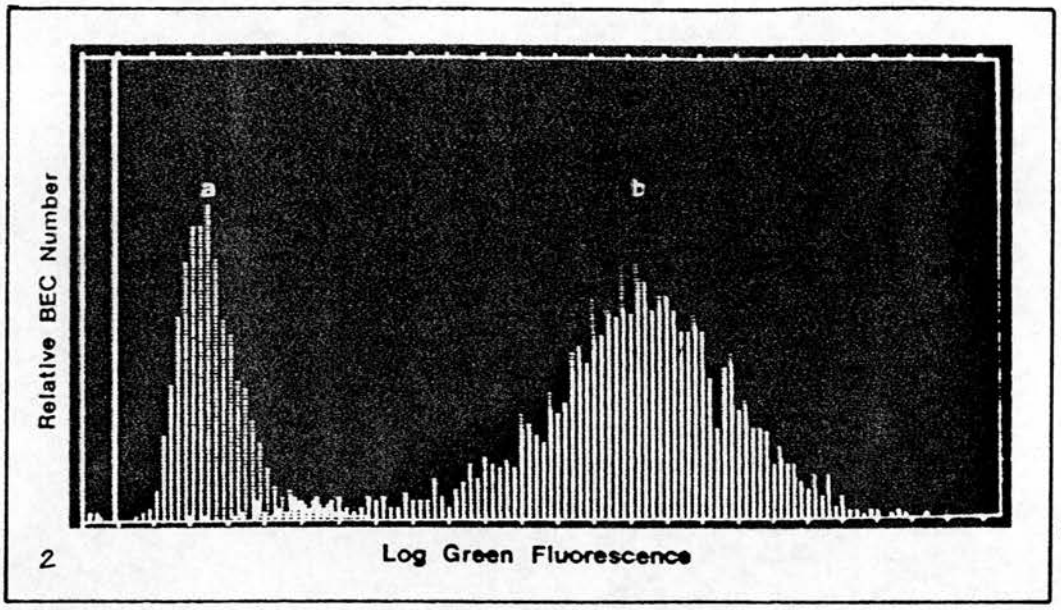


Fig. 2 Flow cytometric comparison of UEAI binding to secretor (b) and non-secretor (a) BEC. Cursor set to give 1% background level.

Rahmen ohne Raster

8 Cic.

There was no significant difference in the amount of H type 2 on BEC of secretors and non-secretors as detected by moAb (fig. 3).

After incubation of BEC from non-secretors with secretor saliva, there was a statistically significant increase ($p<0.005$) in the amount of H antigen detected by UEAI (table 1). There was no significant change in the levels of H when secretors cells were incubated with secretor saliva (table 1). No significant difference was found in the amount of H type 2 antigen on the cells of either secretors or non-secretors after they were incubated with saliva (table 1).

Table 1. Adsorption of H from secretors' saliva onto BEC

	Secretor cells				Non-secretor cells			
	UEAI		anti-H2		UEAI		anti-H2	
	B	A	B	A	B	A	B	A
Mean	710.8	594.5	185.0	173.0	90.3*	246.0*	156.8	157.5
1 SEM	51.8	58.1	23.0	6.6	17.0	22.3	21.8	14.1

Detection of H with UEAI and monoclonal anti-H type 2 on BEC from secretors and non-secretors before (B) and after (A) incubation with secretor saliva. The results represent the mean fluorescence channel±1 SEM of 4 experiments.

* $p<0.005$

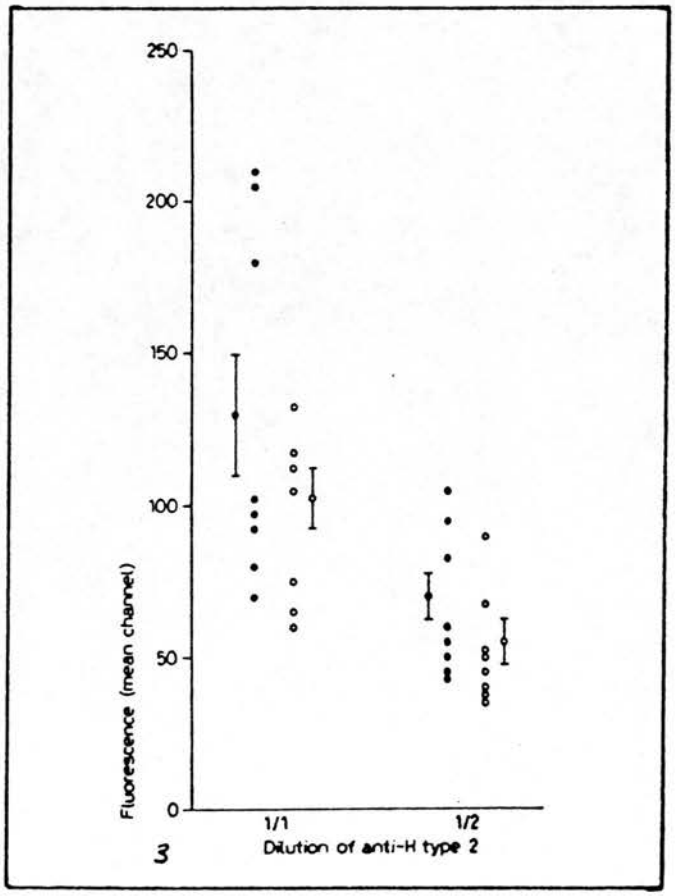


Fig. 3. Determination of H type 2 on BEC from secretors (●) and non-secretors (○). Bars represent mean±1 SEM.

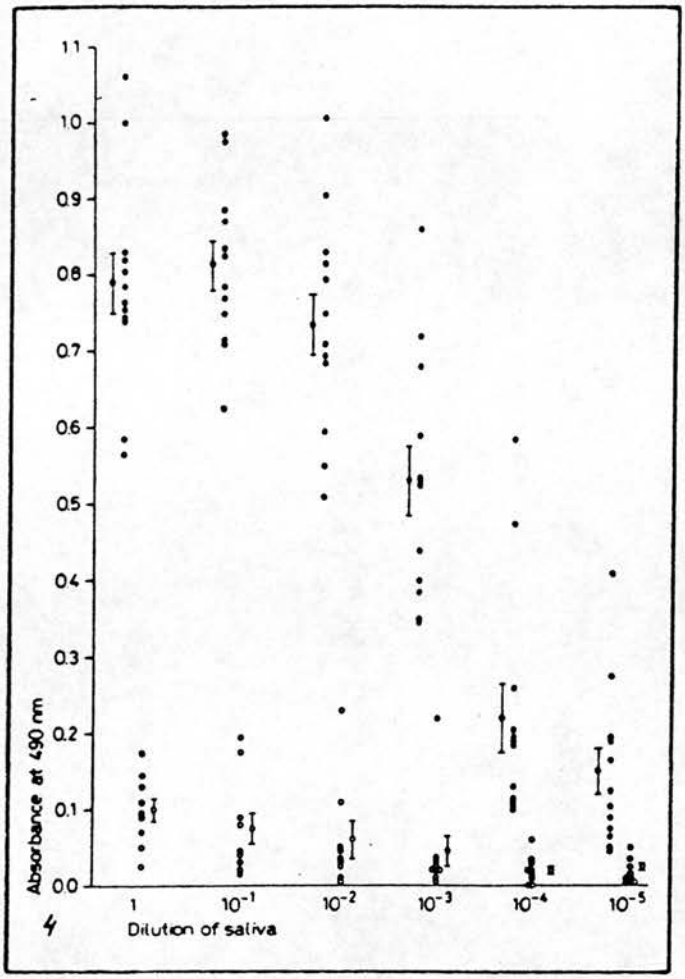


Fig. 4. Measurement of H in saliva from secretors (●) and non-secretors (○) by ELISA. Bars represent mean±1 SEM.

By ELISA, H antigens in saliva of secretors can be detected even at a dilution of 10^5 (fig. 4). In contrast, non-secretors saliva contains significantly lower levels of H ($p<0.001$).

Discussion

Two sensitive methods for the determination of H antigen have been developed in this study. Flow cytometric analysis of BEC with UEAI allows measurement of the total amount of H antigen, and with moAb, the amount of H type 2 antigen can be determined. The biotin streptavidin ELISA can be used to measure the amount of H in saliva.

Previous studies have reported that secretors express more H antigen on their BEC compared with non-secretors [5, 7]. By the method used in this study, we have confirmed this observation; furthermore, the mean fluorescence level, as measured on a linear scale, indicates the amount of antigen found on the cells. By comparing the mean fluorescence for secretors with non-secretors, we have determined the magnitude of this difference.

UEAI detects both H type 2 and H type 1; it can agglutinate red block cells by their H type 2, and this agglutination can be inhibited by H type 1 in secretor saliva. As reported by Hindsgaul et al. [12], this lectin does not react with native H type 1 determinant but with deacetylated H type 1. Therefore, the definition of secretor status is based on deacetylated H type 1 and not the native structure. However, when H type 1 is mentioned in this study, it actually refers to the deacetylated form, since *Ulex europaeus* was used for the detection of H.

According to our results, virtually all BEC from the two populations express the antigen. However, depending on the concentration of UEAI used, secretors have on average between 3.5 and 6 times more H antigen on their BEC surface than non-secretors. In contrast, there was no difference in the amount of H type 2 detected by the moAB on cells of secretors compared with non-secretors. The difference in the level of H on BEC from the two groups appears to be due to H type 1. This observation is in agreement with the view of Oriol et al. [3]. According to this theory, there are two fucosyltransferases responsible for the production of H antigen: one, controlled by the secretor gene, preferentially reacting with type 1 chains, and the other, controlled by the the H gene, preferentially reacting with type 2 chains. Since both secretors and non-secretors express the H gene product, no difference was found in the amount of H type 2 on cells of secretors compared with non-secretors. In this study the increased amount of H on secretors' cells is attributed to H type 1 resulting from the action of the secretor gene product.

Incubation of non-secretor cells with secretor saliva showed that the H antigen can be adsorbed from saliva onto the cell surface. The increase in the amount of H was detected with UEAI and not with anti-H type 2. These results suggest that H type 1 antigen present in secretor saliva is adsorbed onto their cell surface through a mechanism similar to that described for the Lewis antigen [13]. This explains the difference found in the amount of H type 1 between secretors and non-secretors. Although, as suggested by Le Pendu et al. [6], the secretor gene product may be able to fucosylate precursor type 2 chain, the results presented here indicate that the H type 2 antigen is not present

simple means of determining secretor status, providing a quicker and more reliable method than the haemagglutination inhibition test. Using 1/10 dilutions of saliva specimens, the large difference in the amount of H between secretors and non-secretors was clearly demonstrable. The small amount of H detected in non-secretors saliva is probably due to H type 2 antigen derived from epithelial cells or contaminating blood cells.

The methods in this study have been developed in order to investigate the role of blood group antigens as receptors for bacteria. The ELISA was used to investigate the ability of bacteria to adsorb H antigen from suspensions. By flow cytometry, we were able to determine the amount of antigen found on BEC and correlate it with bacterial attachment to BEC (manuscript in preparation).

There are additional applications for this method to studies in oncology. The blood group antigens normally present in the epithelium are reported to be partially or completely lost by neoplastic cells [14–16]. Accurate determination of blood group antigens expressed on cells using the method described in this paper might be useful in early diagnosis of malignancies as previously suggested [17–19].

Acknowledgement

This work was supported by grants from Tenovus-Scotland, the Meningitis Trust and the Medical Research Council. We are grateful to Mrs. Diana Harkiss for technical assistance with the flow cytometer, our colleagues who supplied specimens and Dr. R. H. Fraser and Dr. G. Inglis from the Glasgow, UK, and West of Scotland Blood Transfusion Service for helpful discussions and supplying monoclonal antibodies.

References

1 Blackwell CC: The role of AB0 blood groups and secretor status in host defences. FEMS Microbiol Immunol 1989;47:341–350.
2 Clausen H, Hakomori S: ABH and related histo-blood group antigens: Immunochemical differences in carrier isotypes and their distribution. Vox Sang 1989;56:1–20.
3 Oriol R, Danilous J, Hawkins BR: A new genetic model proposing that the Se gene is a structural gene closely linked to the H gene. Am J Hum Genet 1981;33:421–431.
4 Dabelsteen E, Vendofle P, Hakomori S, et al: Carbohydrate chains specific for blood group antigens in differentiation of human oral epithelium. J Invest Dermatol 1982;79:3–7.
5 Florry LL: Differences in the H antigen on human buccal cells from secretor and non-secretor individual. Vox Sang 1966;11:137–156.
6 Le Pendu J, Lemieux RU, Lambert F, et al: Distribution of H type 1 and H type 2 antigenic determinants in human sera and saliva. Am J Hum Genet 1982;34:402–415.
7 Vedtofte P, Hansen HE, Dabelsteen E: Distribution of blood group antigen H in human buccal epithelium of secretors and non-secretors. Scand J Dent Res 1981;89:188–195.
8 Mollison PL: Red cell grouping techniques; in Mollison PL: Blood Transfusion in Clinical Medicine, Oxford, Blackwell, 1983, pp 492–494.
9 Mackie A, Fraser RH, Allan EK, et al: Further examples of mouse monoclonal anti-H type II. Biotest Bull 1984;2:155–158.
10 Johnson GD, Holborow EJ: Preparation and use of fluorochrome conjugates; in Weir DM (ed): Handbook of Experimental Immunology, Oxford, Blackwell 1986; vol 1, pp 28.4–28.5.
11 Lanier LL, Warner NL: Paraformaldehyde fixation of hematopoietic cells for quantitative flow cytometry (FACS) analysis. J Immunol Methods 1981;47:25–30.
12 Hindsgaul O, Khare DP, Bach M, Lemieux RU: Molecular recognition. 3. The binding of the H-type-2 human blood group determinant by the lectin-1 of *Ulex europaeus*. Can J Chem 1985;63:2653–2658.
13 Marcus DM, Cass LE: Glycosphingolipids with Lewis blood group activity: Uptake by human erythrocytes. Science 1969;164:553–555.
14 Dabelsteen E, Pindborg JJ: Loss of epithelial blood group substance A in oral carcinomas. Acta Pathol Microbiol Scand [A] 1973;81:435–444.
15 Davidsohn J, Kovarik S, Lee CL: A, B and 0 substances in gastrointestinal carcinoma. Arch Path 1966;81:381–390.
16 Prendergast RC, Toto PD, Gargiulo AW: Reactivity of blood group substances of neoplastic oral epithelium. J Dent Res 1968;47:306–310.
17 Dabelsteen E, Fulling HJ: A preliminary study of blood group substances A and B in oral epithelium exhibiting atypia. Scand J Dent Res 1971;79:387–393.
18 Davidsohn J, Kovarik S, Ni LY: Isoantigens A, B and H in benign and malignant lesions of the cervix. Arch Path 1969;87:306–314.
19 Davidsohn J, Ni LY, Stejskal R: Tissue isoantigens A, B and H in carcinioma of the pancreas. Cancer Res 1971;31:1244–1246.

Received: January 2, 1990
Revised manuscript received: February 12, 1990
Accepted: February 12, 1990

J. Stewart
Department of Bacteriology
The Medical School
University of Edinburgh
Edinburgh EH8 9AG (UK)

INFECTION AND IMMUNITY, Nov. 1991, p. 4252–4254
0019-9567/91/114252-03$02.00/0

Vol. 59, No. 11

In Vitro Binding of *Helicobacter pylori* to Human Gastric Mucin

LEONIDAS S. TZOUVELEKIS,[1]* ANDREAS F. MENTIS,[1] ANTONIOS M. MAKRIS,[2] CHRISTOS SPILIADIS,[3] CAROLINE BLACKWELL,[1] AND DONALD M. WEIR[1]

Department of Bacteriology, Hellenic Pasteur Institute, 127 Vassilisis Sofias Avenue,[1] and Department of Internal Medicine, Hippocration Hospital,[2] 11521 Athens, and Department of Gastroenterology, Athens General Hospital, 11527 Athens,[3] Greece

Received 15 October 1990/Accepted 12 August 1991

The in vitro binding of four *Helicobacter pylori* strains to human gastric mucin was studied with an enzyme-linked immunosorbent assay. All four strains were found to bind to purified mucin. Neuraminidase treatment and nonspecific oxidation of mucin decreased bacterial adherence to the macromolecule. Mucin preparations were also found to inhibit attachment of *H. pylori* to HEp-2 monolayers.

The human gastrointestinal tract is covered with a continuous layer of mucus which act as a protective barrier for the underlying mucosa. The main component of mucus is a high-molecular-weight ($>2 \times 10^6$) glycoprotein (mucin). Interactions between bacterial pathogens and mucins derived from humans and animal species have been reported in several studies (8, 11, 13, 15). Colonization of gastric mucosa by *Helicobacter pylori* is associated with chronic active antral gastritis and peptic ulceration (1, 2). The organism can be detected both attached to the gastric epithelial cells and free in the mucous layer (3, 6). In the present work, we studied some characteristics of *H. pylori* binding to human gastric mucin as well as the ability of mucin preparations to inhibit the adherence of the microorganism to HEp-2 cells.

Four *H. pylori* strains—W11, W82, W83, and W114—cultured from gastric-biopsy samples were used. The strains were characterized by colony and Gram stain morphology and production of urease, catalase, and oxidase. Mucosal scrapings of the fundus and pyloric antrum of stomachs obtained postmortem from two individuals with no history of gastrointestinal disease were used for the purification of gastric mucin. Mucin was purified as previously described (16) by equilibrium density gradient centrifugation in CsCl (twice) followed by filtration on a Sepharose 2B column (Pharmacia Fine Chemicals, Uppsala, Sweden). Purified mucin harvested from the void volume fractions of the column was dialyzed against distilled water, lyophilized, quantitated by dry weight, and stored at −70°C. Before use, mucin preparations were dissolved in phosphate-buffered saline (PBS; pH 7.4) at a final concentration of 400 μg/ml. The absence of low-molecular-weight material was confirmed after the final preparation was run on a sodium dodecyl sulfate-polyacrylamide gel (4% stacking gel, 12% resolving gel) under nondenaturing conditions and stained with silver. In addition, 1-mg portions of purified mucin in 2.5 ml of PBS were either treated with 0.5 U of *Clostridium perfringens* neuraminidase type V (Sigma Chemical Co., St. Louis, Mo.) per ml for 15 min at 37°C or oxidized nonspecifically with sodium metaperiodate (5 mg/ml) at 4°C for 30 min in the dark.

Bacterial binding to intact, neuraminidase-treated, and metaperiodate-oxidized mucins and the abilities of these three different mucin forms to inhibit bacterial adherence to HEp-2 cells were evaluated by an enzyme-linked immunosorbent assay (ELISA) utilizing anti-*H. pylori* rabbit serum prepared as previously described (12). A series of preliminary experiments with strain W11 were performed to determine whether ELISA readings corresponded to the number of bacteria on immobilized mucin and on HEp-2 cell monolayers. A good correlation between the ELISA readings and the numbers of bacteria counted microscopically was evident (Fig. 1). Also, in the same experiments, various parameters were probed in order to define the appropriate conditions for the experiments.

Wells of polystyrene microdilution plates (Nunc-Immunoplate Maxisorp; Nunc Inc., Roskilde, Denmark) were coated overnight at 4°C with 100 μl of untreated mucin. Mucin was omitted from control wells. After unbound material was washed off, the remaining sites in the mucin-coated and control wells were blocked with 4% (wt/vol) skim milk for 3 h at room temperature. Serial dilutions of bacteria in PBS were then added (100 μl per well). The initial suspension was adjusted to 10^9 CFU/ml. The plates were incubated for 2 h at 37°C under microaerophilic conditions. Unbound bacteria were removed by four washes with PBS containing Tween 20 (0.05% [vol/vol]). Anti-*H. pylori* immune serum (100 μl, 1/1,000 dilution) was then added. After incubation for 30 min at 37°C, the wells were washed with PBS-Tween 20 and incubated with 100 μl of a 1/2,000 dilution of swine anti-rabbit immunoglobulins conjugated to peroxidase (Dako Immunoglobulins, Glostrup, Denmark) for 30 min. After the washes, 100 μl of an *O*-phenylenediamine solution (0.4 mg/ml) containing 0.4 μl of H_2O_2 (30% [vol/vol]) per ml was added as substrate and incubated at 37°C. After 10 min, the reaction was stopped with 100 μl of 1 N H_2SO_4, and the A_{490} was recorded in a MR5000 MicroELISA Autoreader (Dynatech Laboratories, Inc.).

Differences in bacterial binding to mucin after neuraminidase or metaperiodate treatment were estimated by microaerophilically incubating 5×10^7 PBS-suspended cells per strain with 20 μg of each mucin type for 30 min at 37°C. The total volume was 100 μl. In control wells, 20 μg of gelatin was added. The suspensions were then transferred to the wells of ELISA plates precoated with mucin and were incubated further for 90 min. The degree of binding was evaluated as described above.

The inhibitory effects of mucin preparations on *H. pylori* adherence to HEp-2 cells were examined as follows. HEp-2 cells were grown to confluence in 96-well tissue culture plates (Costar, Cambridge, Mass.). The monolayers were fixed with 0.25% glutaraldehyde for 10 min at 4°C and treated

* Corresponding author.

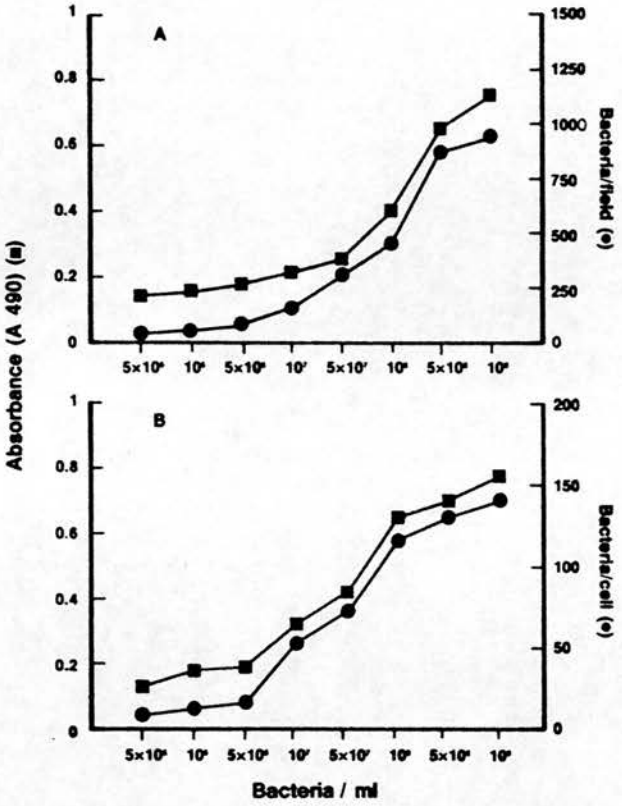


FIG. 1. Correlation of ELISA values with the number of bacteria bound on mucin (A) and attached to HEp-2 cells (B). ELISA was performed as described in the text. For direct microscopic counts, the same procedure was followed except that instead of *O*-phenylenediamine, diaminobenzidine was used as the substate. Bacteria were counted with an inverted microscope (magnification, ×400).

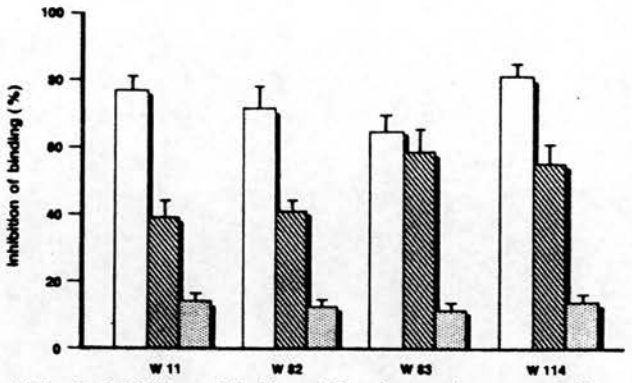


FIG. 2. Inhibition of binding of *H. pylori* strains to mucin immobilized in ELISA microplates by intact mucin (open bars), neuraminidase-treated mucin (hatched bars), and sodium metaperiodate-oxidized mucin (stippled bars). Data are presented as the mean values of five or more assays. Error bars show the standard deviations of mean values.

with 0.1% (wt/vol) gelatin for 1 h at room temperature. Fixation was necessary to retain monolayers intact throughout the multiple washes required for quantitation by ELISA. Twofold dilutions of each mucin form (50 μl) at an initial concentration of 400 μg/ml were placed in the wells. A constant amount of each *H. pylori* strain (approximately 3×10^7 CFU in 50 μl of PBS) was then added. After incubation at 37°C for 2 h under microaerophilic conditions, the plates were washed five times with PBS-Tween 20. To estimate the bacterial adherence, the ELISA procedure described above was followed. After color development was stopped, the liquid content of each well was transferred to ELISA plates and the A_{490} was recorded. The results were analyzed by Student's *t* test.

All four *H. pylori* strains were found to bind to untreated mucin in progressively increasing numbers, reaching saturation when 5×10^7 CFU was added per well. At the saturation point, the mean values for ratios of binding to mucin/binding to blocking agent were 8.1 for strain W11, 7.6 for W82, 7.3 for W83, and 8.4 for W114. Binding curves were essentially the same for strains W11 and W82, showing maximum slopes at between 1×10^7 and 5×10^7 CFU added per well. Binding slopes were approximately the same between 1×10^6 and 5×10^7 CFU per well for both W83 and W114 strains. Binding ratios at the lower dilution used (10^6 CFU per well) were comparable for all strains tested, ranging from 3.2 to 3.8. Each ratio represents the average of 10 measurements not differing by more than 5%.

As shown in Fig. 2, incubation of bacteria with each of the mucin forms in the soluble state decreased bacterial binding to immobilized untreated mucin. The greatest inhibition was observed with homologous mucin. Mean inhibition was substantially less when neuraminidase-treated mucin was used, but the difference was not statistically significant in strain W83. Periodate-oxidized mucin affected binding only slightly, with inhibition percentages never exceeding 18%.

Experiments to assess inhibition of bacterial adherence to HEp-2 cells (Table 1) showed that in the presence of

TABLE 1. Percent inhibition of adherence of four *H. pylori* isolates to HEp-2 monolayers by three mucin types

Strain	Inhibition of bacterial adherence (% inhibition ± SD) at mucin concn of[a]:								
	200 μg/ml			100 μg/ml			50 μg/ml		
	UM	NM	PM	UM	NM	PM	UM	NM	PM
W11	75 ± 13	39 ± 3[b]	9 ± 3[b]	68 ± 5	16 ± 3[b]	5 ± 3[b]	21 ± 8	ND	ND
W82	88 ± 7	52 ± 10[b]	12 ± 1[b]	68 ± 10	24 ± 2[b]	ND	15 ± 4	11 ± 4[c]	ND
W83	62 ± 14	56 ± 4[b]	15 ± 3[b]	44 ± 4	30 ± 6[b]	8 ± 1[b]	9 ± 2	6 ± 1[c]	ND
W110	85 ± 8	55 ± 9[b]	12 ± 2[b]	80 ± 12	28 ± 7[b]	ND	16 ± 5	ND	ND

[a] Percent inhibition was calculated as follows: $[(A_{490}$ of control $- A_{490}$ in the presence of mucin) $\times 100]/A_{490}$ of control). Each value is the average of 10 assays. *P* values were obtained by comparing inhibition caused by untreated mucin with inhibition caused by each of the two forms of treated mucin. UM, untreated mucin; NM, neuraminidase-treated mucin; PM, periodate-oxidized mucin; ND, not detected.

[b] $P < 0.01$.

[c] $P < 0.05$.

untreated mucin, adherence of all *H. pylori* strains was markedly reduced. Increasing concentrations of mucin enhanced the degree of inhibition. Bacterial adherence could be inhibited with as little as 25 μg of mucin per ml. Below this concentration, no inhibition was detected. The inhibitory effect was significantly lower with neuraminidase-treated mucin for all but W83 strains. An even greater loss of inhibitory activity was observed after metaperiodate oxidation of mucin.

Studies on the adherence properties of *H. pylori* have shown it to possess a fibrillar hemagglutinin which recognizes sialic acid-containing structures on erythrocytes (5) and on mouse Y-1 adrenal cells (4). It has also been found that mucins of different origin, such as porcine gastric mucin, bovine submaxillary mucin (10), and human salivary mucin (9), are able to inhibit *H. pylori* hemagglutination activity. Additionally, in the case of human salivary mucins, removal of sialic acid resulted in a substantial decrease in their inhibitory effect. These observations might indicate that outer surface structures of *H. pylori* interact with sialic acid-terminated molecules present on gastric epithelial cells as well as with analogous structures present in soluble form in the gastric mucous layer.

The present study provides evidence that the organism is able to bind to human gastric mucin and that this binding might prevent adherence of the organism to epithelial cells. Sialic acids appear to be partly responsible for this interaction. However, nonspecific oxidation of the sugar moieties of mucin was required to further decrease binding. This indicates that carbohydrate structures other than sialic acids also contribute to the binding.

Although we cannot assign any particular role to the interaction between the microorganism and the gastric mucin, this interaction might protect against *H. pylori* by inhibiting attachment of the organism to the underlying gastric epithelial cells, thus assisting removal of *H. pylori* by the continuous flow of mucus. Alternatively, as has been suggested for other pathogens (7, 8), attachment of *H. pylori* to gastric mucin might facilitate dissemination of the organism in the stomach mucous layer and subsequent colonization of the underlying mucosa.

It is believed that the specific characteristics of mucins and invading microorganisms affect their abilities to interact. Human gastric mucin appears to be altered in patients with certain diseases such as gastritis or peptic ulceration (16). Additionally, as reported by Slomiany et al. (14), *H. pylori* can enzymatically degrade mucin, thus altering its physicochemical properties. Therefore, the detailed study of *H. pylori* interactions with several gastric mucin types may help explain the colonization and pathogenic processes.

REFERENCES

1. **Blaser, E. H.** 1987. Gastric *Campylobacter*-like organisms, gastritis and peptic ulcer disease. Gastroenterology **93**:371–383.
2. **Buck, G. E., W. K. Gourley, W. K. Lee, K. Subramanyam, J. M. Latimer, and A. R. DiNuzzo.** 1986. Relation of *Campylobacter pyloridis* to gastritis and peptic ulcer. J. Infect. Dis. **153**:664–669.
3. **Chen, X. G., P. Correa, J. Offerhaus, E. Rodriguez, F. Janney, E. Hoffman, J. Fox, F. Hunter, and S. Diavolitsis.** 1986. Ultrastructure of the gastric mucosa harboring *Campylobacter*-like organisms. Am. J. Clin. Pathol. **86**:575–582.
4. **Evans, D. G., D. J. Evans, Jr., and D. Y. Graham.** 1989. Receptor-mediated adherence of *Campylobacter pylori* to mouse Y-1 adrenal cell monolayers. Infect. Immun. **57**:2272–2278.
5. **Evans, D. G., D. J. Evans, Jr., J. J. Moulds, and D. Y. Graham.** 1988. *N*-Acetylneuraminyllactose-binding fibrillar hemagglutinin of *Campylobacter pylori*: a putative colonization factor antigen. Infect. Immun. **56**:2896–2906.
6. **Goodwin, C. S., J. A. Armstrong, and B. J. Marshall.** 1986. *Campylobacter pyloridis* gastritis and peptic ulceration. J. Clin. Pathol. **39**:353–365.
7. **Lee, A., J. L. O'Rourke, P. J. Barrington, and T. J. Trust.** 1986. Mucus colonization as a determinant of pathogenicity in intestinal infection by *Campylobacter jejuni*: a mouse cecal model. Infect. Immun. **51**:536–546.
8. **Mantle, M., L. Basaraba, S. C. Peacock, and D. G. Gall.** 1989. Binding of *Yersinia enterocolitica* to rabbit intestinal brush border membranes, mucus, and mucin. Infect. Immun. **57**:3292–3299.
9. **Mentis, A., L. Tzouvelekis, C. Spiliadis, C. C. Blackwell, and D. M. Weir.** 1990. Inhibition of *Helicobacter pylori* haemagglutination activity by human salivary mucins. FEMS Microbiol. Immunol. **64**:125–128.
10. **Nakazawa, T., M. Ishibashi, H. Konishi, T. Takemoto, M. Shigeeda, and T. Kochiyama.** 1989. Hemagglutination activity of *Campylobacter pylori*. Infect. Immun. **57**:989–991.
11. **Nelson, J. W., M. W. Tredgett, J. K. Sheehan, D. Thornton, D. Notman, and J. R. W. Govan.** 1990. Mucinophilic and chemotactic properties of *Pseudomonas aeruginosa* in relation to pulmonary colonization in cystic fibrosis. Infect. Immun. **58**: 1489–1495.
12. **Perez-Perez, G. I., and M. J. Blaser.** 1987. Conservation and diversity of *Campylobacter pyloridis* major antigens. Infect. Immun. **55**:1256–1263.
13. **Ramphal, R., N. Houdret, L. Koo, G. Lamblin, and P. Roussel.** 1989. Differences in adhesion of *Pseudomonas aeruginosa* to mucin glycopeptides from sputa of patients with cystic fibrosis and chronic bronchitis. Infect. Immun. **57**:3066–3071.
14. **Slomiany, B. L., J. Bilski, J. Serosisk, V. L. N. Marty, H. Dworkin, K. VanHorn, J. Zielenski, and A. Slomiany.** 1987. *Campylobacter pyloridis* degrades mucin and undermines gastric mucosal integrity. Biochem. Biophys. Res. Commun. **14**: 307–314.
15. **Wanke, C. A., S. Cronan, C. Goss, K. Chadee, and R. L. Guerrant.** 1990. Characterization of binding of *Escherichia coli* strains which are enteropathogens to small-bowel mucin. Infect. Immun. **58**:794–800.
16. **Younan, F., J. Pearson, A. Allen, and C. Venables.** 1982. Changes in the structure of the mucous gel on the mucosal surface of the stomach in association with peptic ulcer disease. Gastroenterology **82**:827–831.

Epidemiol. Infect. (1991), **106**, 221–229
Printed in Great Britain

ABO blood group, secretor status and detection of *Helicobacter pylori* among patients with gastric or duodenal ulcers

A. MENTIS[1], C. C. BLACKWELL[2]*, D. M. WEIR[2], C. SPILIADIS[3], A. DAILIANAS[3] AND N. SKANDALIS[3]

[1] *Hellenic Institute Pasteur, Athens*
[2] *Department of Medical Microbiology, The Medical School, University of Edinburgh, Edinburgh*
[3] *Department of Gastroenterology, General Hospital of Athens, Athens*

(*Accepted 5 November 1990*)

SUMMARY

Patients (454) referred for gastroscopy to the General Hospital of Athens were examined to determine (1) if non-secretors were over-represented among patients with ulcers and (2) is there was an association with ABO blood group or secretor status and carriage of *Helicobacter pylori*.

Compared with the local population, among patients with either gastric ulcer (51) or duodenal ulcer (96) there was a significant increase in the proportion of those who were blood group O ($P < 0{\cdot}025$); however, there were no significant differences in the proportions of non-secretors. *H. pylori* was identified in 62% of the 454 patients: 59·5% of those without evidence of ulcers; 62·5% of those with gastric ulcer; 88% of those with duodenal ulcer ($P < 0{\cdot}0005$). These bacteria were cultured more often and in higher numbers from patients with duodenal ulcer ($P < 0{\cdot}025$). There was no association between ABO blood group and prevalence of *H. pylori*. The prevalence of *H. pylori* among non-secretors with gastric ulcer (12·5%) was significantly lower than that for non-secretors with duodenal ulcer (100%) ($P < 0{\cdot}0005$). This was not observed for secretors.

INTRODUCTION

There are reports from the 1950s and 1960s that individuals of blood group O and those who are non-secretors of the glycoprotein form of their ABO blood group antigens are over-represented among patients with gastric or duodenal ulcers (reviewed by Mourant and colleagues) [1]. Non-secretion is associated with susceptibility to a number of infectious diseases (reviewed by Blackwell) [2, 3] and with asymptomatic carriage of pathogenic bacteria [4, 5] and yeasts [6, 7]. Non-secretors are also over-represented among patients with several autoimmune diseases for which infectious aetiologies have been postulated [4–11].

A recent review summarized the current evidence implicating *Helicobacter pylori* in the pathogenesis of gastroduodenal disease [12]. There is serological evidence

* Corresponding author.

that the majority (approximately 70%) of the adult Greek population (> 25 years of age) has been exposed to *H. pylori* [13]. In this study we examined ABO blood groups and secretor status (by means of Lewis phenotype) of patients referred for gastroscopy to determine: (1) if we could confirm the findings of earlier studies of associations between ABO phenotype and/or non-secretion and ulcers in a European population in which there is a high prevalence of *H. pylori*: and (2) to test the hypothesis that there might be an association with ABO blood group or secretor status and carriage of these bacteria.

SUBJECTS AND METHODS

The study population consisted of 454 consecutive patients between the ages of 17 and 86 referred for gastroscopy to the Gastroenterology Department of the General Hospital of Athens for investigation of symptoms of upper gastrointestinal disease. The majority of the patients had upper abdominal pain (58%), bleeding (melaena or haematemesis) (18%) or anaemia (11%). For men, anaemia was defined as red blood cell (RBC) count < 4500000/ml, haemoglobin (Hb) < 14 g/dl and haematocrit (HCT) < 42%. For women, anaemia was defined as RBC count < 4000000/ml, Hb < 12 g/dl and HCT < 37%.

For this study, during gastroscopy, three biopsy specimens were taken from the pylorus. Two specimens for culture were placed in separate containers with 1 ml of thioglycolate broth under aseptic conditions. A third specimen was fixed in 10% formalin, processed in paraffin and 3 μm sections were cut. The sections were stained with haematoxylin and eosin and examined by light microscopy for the presence of *H. pylori*.

Before culture, 0·8 ml of the broth was removed from the biopsy material and the specimen was vortexed in the remaining 0·2 ml with 4 or 5 glass beads. The material (0·1 ml) was then plated onto Skirrow medium composed of blood agar base, 7% horse blood, vancomycin (10 μg/ml), trimethoprim (5 μg/ml) and polymyxin B (2·5 IU/ml). Another 0·1 ml was plated onto medium containing Columbia blood agar base, 7% horse blood, and *Campylobacter pylori* supplement of vancomycin (10 μg/ml), trimethoprim (5 μg/ml), cefsulodin (5 μg/ml) and amphotericin B (5 μg/ml). The plates were incubated in microanaerobic conditions (Biomerieux system) for 7 days. The numbers of colonies per plate with characteristic appearance of *H. pylori* were counted and the numbers scored as follows: no growth = 0; 1–20 colonies = 1; 21–100 colonies = 2; 101–200 colonies = 3; > 200 colonies = 4. Colonies with characteristic appearance of *H. pylori* were examined by Gram stain and tested for production of oxidase, catalase and urease. A patient was classified as positive for *H. pylori* if the organism was identified either by culture or in the stained biopsy.

ABO blood group for each patient was determined by slide agglutination with monoclonal anti-A and anti-B antibodies (Scottish National Blood Transfusion Service) and the Lewis blood group phenotype by standard tube agglutination with monoclonal anti-Lewis^a and anti-Lewis^b antibodies (Scottish National Blood Transfusion Service). Individuals who are non-secretors can express only Lewis^a (Le^a) and those who are secretors can express Lewis ^{b} (Le^b). There is no association between sex, age and expression of ABO or Lewis phenotypes in adults. The

results for the patient population were compared with those obtained with the same reagents for a series of 1248 individuals attending the analytical laboratory of the Hellenic Institute Pasteur. These were predominantly blood specimens taken for antenatal screening for antibodies to rubella and toxoplasma. The proportion of ABO phenotypes was similar to those reported previously for Greek populations [14, 15]. The proportions of Le^a and Le^b individuals in the patients attending the Institute were similar to the proportions of Le^a and Le^b individuals identified in a separate study of 891 male military results (Blackwell, unpublished results).

Epidemiological data, sex, age, symptoms, medication, smoking, alcohol consumption, results of biopsy reports, gastroscopy and culture results were coded and stored in a Data Base III plus data base. Results were analysed with the 'EXPLORE' statistical package, χ^2 test incorporating Yates' correction factor or student's t test.

RESULTS

Patients referred for gastroscopy

Of the 454 individuals (age range 17–86 years, mean age = 52 years) referred for gastroscopy, 60% were men (age range 18–86 years, mean age = 52 years) and 40% were women (age range 17–83 years, mean age = 51 years). There were 162 smokers (36%); 126 (78%) were men and 36 (22%) were women. The proportion of men who smoked was 126/270 (47%) and of women who smoked 36/181 (20%).

The proportion of individuals in whom *H. pylori* was identified by culture or by stained biopsy was 286 (68%). The proportions of men and of women in whom *H. pylori* was identified were 68 and 67% respectively. There were no significant associations when presence of the bacteria was analysed with reference to sex and smoking or sex and alcohol use.

The distribution of the ABO blood groups and Lewis phenotypes of the patient population is compared with that of the control population in Table 1.

Patients with ulcers

Results of gastroscopy identified 51 patients with gastric ulcer and 96 with duodenal ulcer. Among patients with gastric ulcer there were 31 men (61%) and 20 women (39%). Among the patients with duodenal ulcer, there were 78 men (81%) and 18 women (19%) which differed significantly compared with the total study population ($\chi^2 = 14{\cdot}9$, D.F. = 1, $P < 0{\cdot}0005$). For both men and women, the mean age was significantly higher among the patients with gastric ulcers compared to those with duodenal ulcers (Table 2).

Presence of ulcer was analysed by sex and smoking habits and by sex and alcohol consumption. No correlation was found with either of these factors.

Among patients with ulcers the proportion of those of blood group O was significantly increased compared with other blood groups (not O) (Table 3). Compared with the proportion of group O in the control population (40%), there were 29 patients of group O among the 51 patients with gastric ulcer (57%) ($\chi^2 = 5{\cdot}4$, D.F. = 1, $P < 0{\cdot}025$) and 51 of group O among the 96 patients with duodenal ulcer (53%) ($\chi^2 = 6{\cdot}2$, D.F. = 1, $P < 0{\cdot}025$). The proportion of patients with gastric

Table 1. *ABO and Lewis phenotypes of patient population and controls*

ABO group	Patients No.	Patients (%)	Controls No.	Controls (%)
A	165	(36)	513	(41)
B	52	(11)	183	(15)
O	217	(48)	494	(40)
AB	20	(4)	58	(4)
Lewis phenotypes				
Le^{a+b-}	93	(21)	249	(20)
Le^{a-b+}	356	(78)	964	(77)
Le^{a-b-}	5	(1)	32	(3)

Table 2. *Ages of patients with gastric or duodenal ulcers*

Sex	Site of ulcer	Age range	Mean age
Men	Gastric	22–78	56*
	Duodenal	22–75	47
Women	Gastric	34–77	62†
	Duodenal	30–71	52

* $t = 2{\cdot}153$. D.F. = 105. $P < 0{\cdot}025$.
† $t = 2{\cdot}460$. D.F. = 36. $P < 0{\cdot}01$.

Table 3. *Distribution of ABO groups among patients with ulcers*

Blood group	Gastric ulcer ($n = 51$) %	Duodenal ulcer ($n = 96$) %	Control ($n = 1248$) %
A	31	35	41
B	10	10	15
O	57	53	40
AB	2	1	4

Gastric ulcer O/NOT O. $\chi^2 = 5{\cdot}4$. $P < 0{\cdot}025$
Duodenal ulcer O/NOT O. $\chi^2 = 6{\cdot}2$. $P < 0{\cdot}025$.

ulcer expressing Le^a was 16% and those expressing Le^b was 84%. Among those with duodenal ulcer, the proportion expressing Le^a was 27% and those expressing Le^b 73%. The figures for Lewis phenotypes did not differ significantly from those of the controls (Table 1).

There were 301 individuals in whom ulcers were not detected and for whom results of both culture and biopsy were available. *H. pylori* was identified in 179 (59·5%). The bacteria were identified in 30 of 48 (62·5%) patients with gastric ulcer for whom results of both culture and biopsy were available. It was identified among 82 of 93 (88%) patients with duodenal ulcer. The prevalence of the bacteria among the patients with duodenal ulcers was significantly higher than that of the study group ($\chi^2 = 19{\cdot}5$, D.F. = 1, $P < 0{\cdot}0005$).

Among the patients in whom no ulcer was found, *H. pylori* was identified in 46% of those who were group O and 54% of those who were not group O. *H. pylori* was identified in 64% of group O patients with gastric ulcer compared with 60%

Table 4. *Quantitative isolation of* H. pylori *from patients without ulcers or gastric or duodenal ulcer*

Gastroscopy result	No growth	No. bacteria per biopsy			
		1–20	21–100	101–200	⩾ 200
No ulcer (n = 308)	153 (50%)	40 (13%)	36 (12%)	41 (13%)	38 (12%)
Gastric ulcer (n = 48)	22 (46%)	9 (19%)	6 (12%)	8 (17%)	3 (6%)
Duodenal ulcer (n = 93)	22 (24%)	20 (22%)	6 (6%)	23 (25%)	22 (24%)

No ulcer/duodenal ulcer $\chi^2 = 28{\cdot}37$, D.F. = 4, $P < 0{\cdot}0005$.
Gastric ulcer/duodenal ulcer $\chi^2 = 12{\cdot}81$, D.F. = 4, $P < 0{\cdot}025$.

among those who were not group O. The bacteria were identified among 92% of patients with duodenal ulcer who were group O compared with 88% of those who were not group O.

Among patients without ulcers, 34 of 59 non-secretors (58%) and 145 of 242 secretors (60%) were positive for the bacteria by either culture or biopsy. Analysis of the prevalence of *H. pylori* among non-secretors and secretors with gastric ulcer revealed significant differences; the bacteria were identified in only 1 of the 8 (12·5%) Le^a/non-secretors but in 28 of the 42 (67%) Le^b/secretors ($\chi^2 = 7{\cdot}5$, D.F. = 1, $P < 0{\cdot}01$). A similar analysis of the prevalence of *H. pylori* among non-secretors and secretors with duodenal ulcer revealed a different pattern; the bacteria were found in all the 24 non-secretors but in only 56 of the 67 (84%) secretors ($\chi^2 = 3{\cdot}1$, $P > 0{\cdot}05$).

Among patients who were non-secretors, the difference in the prevalence of the bacteria between those with gastric ulcer (12·5%) was significantly lower than that for this group with duodenal ulcer (100%) ($\chi^2 = 22{\cdot}0$, $P < 0{\cdot}0005$). Similar analysis of secretors did not find a significant difference in the prevalence of *H. pylori* among patients with gastric ulcer (67%) compared with those with duodenal ulcer (81%) ($\chi^2 = 1{\cdot}5$, $P > 0{\cdot}05$).

H. pylori was cultured more frequently from patients with duodenal ulcer (76%) than those with gastric ulcer (54%) or those without ulcers (50%). The proportions of those with 100 or more colony-forming units per biopsy specimen was 49% for those with duodenal ulcer compared with 23% for those with gastric ulcer ($\chi^2 = 12{\cdot}81$, D.F. = 4, $P < 0{\cdot}025$) of 25% for those without ulcers ($\chi^2 = 28{\cdot}37$, D.F. = 4, $P < 0{\cdot}0005$) (Table 4).

DISCUSSION

The present study confirms the earlier report from Athens [14] that individuals of blood group O are significantly over-represented among patients with either gastric or duodenal ulcers. The proportion of group O subjects in the population referred to the Institute Pasteur for analyses was 40% which is very similar to the figures reported by Hirszfeld and Hirszfeld (42%) in 1919 [15] and Merikas and colleagues (41%) in 1966 [14]. Compared with patients of the other blood groups

(not O), there was a significant increase in the proportion of group O individuals among patients with gastric (57%) or duodenal (53%) ulcers ($P < 0{\cdot}025$). In contrast to earlier findings for both British and Greek patients with ulcers [14, 16], there was not a significant association between group O and bleeding ulcers compared with non-bleeding ulcers. The number of patients in the present study was, however, smaller than those examined in the previous studies.

In this group of patients we were not able to confirm the reported associations for non-secretion and gastric ulcers (3/10 studies) or duodenal ulcers (13/15 studies (analysed by Mourant and colleagues) [1]. The proportion of Le^a/non-secretors in the population referred to the Institute for analyses was 20%, similar to that found in a separate study of 891 military recruits (21%) (Blackwell, unpublished results). The proportion of Le^a/non-secretors was 16% among patients with gastric ulcers and 27% among those with duodenal ulcers.

The original association of peptic ulcers with the presence of *H. pylori* has been confirmed by some investigators but not by others (reviewed by Buck) [12]. Although cigarette smoking has been identified as a risk factor for ulcers [17] and smoking is associated with carriage of some microorganisms [5], there was no association between smoking and presence of *H. pylori*. The proportion of patients with gastric ulcers in whom *H. pylori* was identified (62·5%) did not differ significantly from that of individuals in whom ulcers were not detected (59·5%) or the prevalence of antibodies to these bacteria in the general population of similar age. Among 458 blood donors (age range 21–50), 70% had serological evidence of exposure to *H. pylori* [13]. Among the patients in the study all but nine were 21 years of age or older and the proportion of males (60%) to females (40%) was the same as the blood donors. The prevalence of *H. pylori* among patients with duodenal ulcers (88%) was significantly increased compared with that of the study population ($P < 0{\cdot}0005$).

The results of this study suggest that in Greece where a high proportion of the population is exposed to *H. pylori* (1) there is not a significant association between the presence of these bacteria and gastric ulcers; (2) the numbers of *H. pylori* isolated from patients with gastric ulcers did not differ significantly from those isolated from patients without ulcers (Table 4). It has been reported that if other causes of gastric ulcer such as the use of the analgesics are excluded, the prevalence of *H. pylori* among patients with gastric ulcer approaches 100% [18]. In the present study only 9 patients were taking high doses of aspirin; 4 of these had gastric ulcers and *H. pylori* was isolated from 2.

The increased prevalence of *H. pylori* among patients with duodenal ulcers and the larger numbers of *H. pylori* isolated from these patients (Table 4) suggest the bacteria might contribute to development of the disease condition. These observations, the lower mean age of both men ($P < 0{\cdot}025$) and women ($P < 0{\cdot}01$) with duodenal ulcer (Table 2) and the lower proportion of women with duodenal ulcer ($P < 0{\cdot}0005$) suggest that a more detailed investigation of epidemiological factors influencing carriage of *H. pylori* in this population might be of value.

No association between presence of *H. pylori* and blood group O was found in patients without ulcers or those with ulcers. The proportion of non-secretors with gastric ulcers in whom the bacteria were found (12·5%) did not differ significantly from the proportion of non-secretors found in the group with gastric ulcers (16%);

but, the proportion of non-secretors with duodenal ulcers in whom bacteria were found (100%) was significantly increased compared with the proportion of non-secretors among those with duodenal ulcers (27%).

One of the hypotheses suggested to explain the increased susceptibility of non-secretors to particular diseases is that their mucosal surfaces might be more readily colonized by the causative microorganisms [2,3]. Non-secretors are significantly over-represented among healthy asymptomatic carriers of *Streptococcus pyogenes* [4], *Neisseria meningitidis* [5] or *Candida albicans* [6,7]. In the study by Burford-Mason and colleagues [6], non-secretion was associated with long-term carriage of the yeast. If *H. pylori* persists for longer periods or is present in larger numbers in the gastrointestinal tract, this might contribute to the pathogenic processes leading to ulcers. There were 24 patients in the present study who were re-examined by gastroscopy because symptoms had recurred or they had not responded to treatment. At the time of gastroscopy, none was being treated with bismuth and only one was taking an antibiotic. *H. pylori* was found in 4 of the 5 (80%) non-secretors but only 7 of the 19 (37%) secretors. The proportion of patients with > 100 colony forming units of *H. pylori* was significantly higher among the patients with duodenal ulcers; however, the numbers of patients were too small for statistical analysis by secretor status.

Several studies have reported that the epithelial cells of non-secretors bind larger numbers of some microorganisms than cells of secretors: uropathogenic strains of *Escherichia coli* [19]; *C. albicans* [20,21]; and meningococci [unpublished results]. Although bacteria can bind to proteins on epithelial surfaces, carbohydrates appear to be the receptors recognized by many bacterial adhesins, probably due to their abundance and variety [22]. There is evidence that blood group antigens act as receptors for several microorganisms. The P blood group antigen is a receptor for some uropathogenic strains of *E. coli* [23]; and the Duffy blood group antigen acts as a receptor for the malaria parasite *Plasmodium knowlesi* [24].

Two blood group antigens common to most individuals have been proposed to act as receptors for microorganisms, H and Le^a [2,3]. H, the antigen of blood group O, is found on the cells of all individuals except the very rare Bombay phenotype [25]. The Lewis antigens on epithelial cells are adsorbed from secretions and reflect those present in the body fluids. Although secretors express Le^b predominantly, some can have substantial amounts of Le^a in their body fluids, and consequently on their epithelial cells [26,27]. The amount of Le^a present in secretors depends on the efficiency of the fucosyl transferase coded for by the secretor gene [28].

Previous studies by our group have found that buccal epithelial cells of secretors express 3–6 times more H than those of non-secretors [29]. If H were one of the receptors for microorganisms, there should be increased attachment to cells of secretors. A second piece of evidence suggests H is not a receptor for *H. pylori*. A gastric glycolipid to which *H. pylori* binds is also found on red blood cells of groups, A, B and O; however, this substance is not one of the ABO blood group glycolipids [30].

There is evidence that glycoproteins containing sialic acids in their sugar moieties can inhibit binding of *H. pylori* to erythrocytes. The carbohydrates are

suggested to bind a fibrillar haemagglutinin [31]. The inhibitory substances are found in porcine gastric mucin, to a lesser extent in bovine submaxillary mucin [32] and in human saliva [33]. Neuraminidase treatment of human saliva reduced its ability to inhibit haemagglutination. This indicated that sialic residues contribute to, but are not totally responsible for, the inhibitory effect of the salivary mucin [33]. The role of Lewis antigens in these interactions is under investigation.

At present little is known about the distribution or amounts of the Le^a antigen on epithelial cells in the stomach and duodenum. Although non-secretors cannot produce Le^b, secretors can have varying amounts of Le^a in body fluids. Quantitative differences in the amount of Le^a present in mucus of secretors and non-secretors of patients with ulcers compared to those without ulcers have not been determined. These and other studies are currently underway to test the hypothesis that Le^a might be one of the receptors for *H. pylori* in the gastrointestinal tract.

ACKNOWLEDGEMENTS

We are grateful to Mrs F. Karafotis, Miss G. Volondaki, Miss A. Tabaki and Mr G. Boursinos for technical assistance and to Mrs M. Cole for preparation of the manuscript.

REFERENCES

1. Mourant AE, Kopec AC, Domaniewska-Sobczak K. Blood groups and diseases. Oxford: Oxford University Press, 1978.
2. Blackwell CC. The role of ABO blood groups and secretor status in host defences. FEMS Microbiol Immunol 1989; **47**: 341–50.
3. Blackwell CC. Genetic susceptibility to infectious agents Proc Roy Coll Physic (Edinburgh) 1989; **19**: 129–38.
4. Haverkorn MJ, Goslings WRO. Streptococci, ABO blood groups and secretor status. Am J Hum Genet 1969; **21**: 360–75.
5. Blackwell CC, Weir DM, James VS, et al. Secretor status, smoking and carriage of *Neisseria meningitidis*. Epidemiol Infect 1990; **104**: 203–9.
6. Burford-Mason AP, Welsby JCP, Willoughby JMT. Oral carriage of *Candida albicans*, ABO blood group and secretor status in healthy individuals. J Med Vet Mycol 1988; **26**: 49–56.
7. Blackwell CC, Aly FZM, James VS, et al. Blood group, secretor status and oral carriage of *Candida* and other fungal species among patients with diabetes mellitus. Diabet Res 1989; **12**: 101–4.
8. Andersen J, Lauritzen E. Blood groups and diabetes mellitus. Diabetes 1960; **9**: 20–4.
9. Blackwell CC, James VS, Weir DM, et al. Secretor status of patients with insulin dependent or non-insulin dependent diabetes mellitus. Br Med J 1987; **295**: 1024–5.
10. Collier A, Patrick AW, Toft AD, Blackwell CC, James VS, Weir DM. Increased prevalence of non-secretors in patients with Graves' disease – evidence for an infective aetiology. Br Med J 1988; **296**: 1162.
11. Shinebaum R, Blackwell CC, Forster PJG, Hurst NP, Weir DM, Nuki G. Non-secretion of ABO blood group antigens as a host susceptibility factor in the spondylarthropathies. Br Med J 1987; **294**: 208–10.
12. Buck GE. *Campylobacter pylori* and gastric duodenal disease. Clin Microbiol Rev 1990; **3**: 1–12.
13. Pateraki E, Mentis A, Spiliadis C, et al. Seroepidemiology of *Helicobacter pylori* infection in Greece. FEMS Microbiol Immunol 1990; **64**: 129–36.
14. Merikas G, Christakopoulos P, Petropoulos E. Distribution of ABO blood group in patients with ulcer disease; its relationship to gastroduodenal bleeding. Am J Digest Dis n.s. 1986; **11**: 790–5.

15. Hirszfeld L. Hirszfeld H. Serological differences between the blood of different races. The result of researches on the Macedonian front. Lancet 1919; ii: 675–9.
16. Langman MJS. Doll R. ABO blood group and secretor status in relation to clinical characteristics of peptic ulcers. Gut 1965; **6**: 270–3.
17. McCarthy DM. Smoking and ulcers – time to quit. N Engl J Med 1984; **311**: 726–8.
18. Rauws EIAJ. Longenberg W. Howthoff HJ. Zanen HC. Tytgat GNJ. *Campylobacter pyrloridis*-associated chronic antral gastritis. Gastroenterol 1988; **94**: 33–40.
19. Lomberg H. Cedergren B. Leffler H. Nilsson B. Carlstrom AS. Svanborg-Eden C. Influence of blood group on the availability of receptors for attachment of uropathogenic *Escherichia coli*. Infect Immun 1986; **51**: 919–26.
20. May SJ. Blackwell CC. Weir DM. Lewis^a blood group antigen. a receptor for *Candida* species. FEMS Microbiol Immunol 1989; **47**: 407–10.
21. Darwazeh AMG. Lamey P-J. Samaranayake LP. et al. The relationship between colonisation of secretor status and *in vitro* adhesion of *Candida albicans* to buccal epithelial cells from diabetics. J Med Microbiol 1990; **33**: 43–50.
22. Lark D. Protein–carbohydrate interactions in biological systems. London: Academic Press. 1986.
23. Kallenius G. Mollby R. Svensson SB. et al. The pk antigen as receptor for the haemagglutinin of pyelonephritic *Escherichia coli*.. FEMS Microbiol Lett 1980; **7**: 297–302.
24. Miller LH. Mason SJ. Dvorak JA. McGinniss MH. Rothman IK. Erythrocyte receptors for (*Plasmodium knowlesi*) malaria: Duffy blood group determinants. Science 1975; **189**: 561–3.
25. Race RR. Sanger R. Blood groups in man. 6th ed.. Oxford: Blackwell Scientific Publications. 1975.
26. Marcus DM. Cass LE. Glycosphingolipids with Lewis blood group activity: uptake by human erythrocytes. Science 1969; **164**: 553–5.
27. Ogata M. Nakasoro I. Iwasaki M. Two familial cases of dissociation of saliva Le^a levels and erythrocytes Lewis type. Hum Hered 1988; **38**: 303–7.
28. Oriol R. Danilous J. Hawkins BR. A new genetic model proposing that the *Se* gene is a structural gene closely linked to the H gene. Am J Hum Genet 1981; **33**: 421–31.
29. Rahat A. Stewart J. Blackwell CC. Weir DM. Semi-quantitative determination of H type 1 and type 2 antigens on buccal epithelial cells and in saliva of secretors and non-secretors. Vox Sang 1990; **59**: 101–5.
30. Lingwood CA. Law H. Pellizzari A. Sherman P. Drumm B. Gastric glycerolipid as a receptor for *Campylobacter pylori*. Lancet 1989; ii: 238–41.
31. Evans DG. Evans DJ. Moulds JJ. Graham DY. N-acetyl-neuraminyl-lactose-binding fibrillar hemagglutinin of *Campylobacter pylori*: a putative colonization factor antigen. Infect Immun 1989; **56**: 2896–906.
32. Nakazawa T. Ishibashi M. Konishi H. Takemoto T. Shigeeda M. Kochiyama T. Haemagglutination activity of *Campylobacter pylori*. Infect Immun 1989; **57**: 989–91.
33. Mentis A. Tzouvelekis L. Spiliadis C. Blackwell CC. Weir DM. Inhibition of *Helicobacter pylori* haemagglutination activity by human salivary mucin. FEMS Microbiol Immunol 1990; **64**: 125–8.

Epidemiol. Infect. (1991), **106**, 355–363
Printed in Great Britain

Chronic atrophic oral candidiasis among patients with diabetes mellitus – role of secretor status

F. Z. ALY[1], C. C. BLACKWELL[1], D. A. C. MacKENZIE[1], D. M. WEIR[1], R. A. ELTON[2], C. G. CUMMING[3], J. A. SOFAER[3] AND B. F. CLARKE[4]

[1] *Department of Medical Microbiology, University of Edinburgh Medical School, Edinburgh EH8 9AG*
[2] *Medical Statistics Unit, University of Edinburgh*
[3] *Department of Oral Medicine and Oral Pathology, University of Edinburgh*
[4] *Department of Diabetes, Royal Infirmary, Edinburgh*

(*Accepted 28 September 1990*)

SUMMARY

Non-diabetic individuals who are non-secretors of blood group antigens are prone to superficial infections by *Candida albicans*. In this study, 216 patients with diabetes mellitus who were denture wearers were examined for the presence or absence of denture stomatitis. There was an overall trend for non-secretors to be prone to denture stomatitis compared with secretors. Stepwise linear discriminant analysis was used to dissect the contribution of secretor status and other variables to the development of the disease. Secretor status was found to be a contributory factor among patients with non-insulin dependent diabetes but not among those with insulin-dependent diabetes. The possible reasons for this are discussed.

INTRODUCTION

Chronic atrophic oral candidiasis (denture stomatitis) is solely associated with the wearing of dentures. The studies of Butdz-Jörgensen [1] and Cawson [2] established the link between *Candida* sp. and denture stomatitis. Yeasts were recovered from over 90% of the lesions and antifungal treatment usually led to regression of the lesion. Subsequently, numerous studies have examined the prevalence of and predisposing factors to denture stomatitis [3]. Presence and continuous wearing of dentures at night, availability of sucrose in the oral environment through dietary intake, traumatic effects of the denture, antibiotics and corticosteroid treatment, diabetes mellitus and smoking have all been implicated as potential factors influencing the development of denture stomatitis.

The genetic inability to secrete ABO blood group antigens in body fluids has been associated with a variety of infectious diseases [4]. Non-diabetic individuals and pregnant women who are non-secretors are prone to superficial candida infections [5]. Non-secretors are also over-represented among carriers of *Candida albicans* in normal subjects and patients with non-insulin dependent diabetes mellitus (NIDDM) [6, 7].

The aims of the present study were:

(1) To assess the influence of secretor status in the development of denture stomatitis (DS) among patients with insulin-dependent diabetes (IDDM) and those with non-insulin dependent diabetes (NIDDM).

(2) To assess the contributions of the following variables to the development of DS: age; sex; duration of diabetic state; type of diabetes; control of diabetes measured by glycosylated haemoglobin (HBA_1); diabetic complications – retinopathy, neuropathy and nephropathy; antibiotics; corticosteroid treatment; smoking; alcohol; presence of dentures at night; type of denture; denture fit, extension, occlusion, age and hygiene; presence of persistent glycosuria and albuminuria; and history of superficial candida infections.

MATERIALS AND METHODS

Subjects

A total of 439 subjects attending for routine follow up examination at the Diabetic Outpatient Department (DOPD), Royal Infirmary Edinburgh were sampled. An initial pilot study examined 80 individuals and was followed by a study that sampled 359 individuals between September 1988 and March 1989. The method of selection was stratified random selection according to sex and type of diabetes.

Clinical history

Each subject was classified as insulin dependent (IDDM) or non-insulin dependent (NIDDM) according to the clinical history of onset, requirement for insulin and progression of the disease. Of the 439 subjects sampled, three could not be classified.

A full medical history including the presence of diabetic complications (retinopathy, neuropathy and nephropathy) was obtained during interview and from the patients' records. A history of medications, with particular reference to antibiotics or corticosteroid-containing preparations, within the past 6 months was noted. A social history of alcohol consumption and smoking was recorded. Subjects were questioned about history of superficial infections due to candida. Glycosuria and albuminuria were recorded as persistent if subjects had positive urine samples on more than two consecutive appointments at the DOPD. None of the subjects used any oral preparations containing antiseptics within the previous 6 months.

Clinical examination

A thorough oral examination of both soft and hard tissues was carried out by the same examiner (FZA). Any of the following abnormalities were noted: gingivitis, periodontitis, angular cheilitis, leukoplakia, median rhomboid glossitis; fissured, geographic or hairy tongue; and denture stomatitis. Denture stomatis has the characteristic appearance of chronic erythema of the portion of the palate underlying the denture. The inflammation is generally diffuse, but may be associated with fibrous hyperplasia of the palate giving it a granular appearance. The occlusion, fit, extension and hygiene of a denture where present was recorded

as 'good' or 'poor'. The age of the denture was recorded as well as whether it was left out of the mouth at night.

Samples

(1) Venous blood was obtained for ABO blood grouping and Lewis antigen determination. Analysis for glycosylated haemoglobin (HBA_1) and random plasma glucose carried out routinely were recorded.

(2) Swabs were obtained from five sites of the mouth including the palate and inoculated immediately into malt broth.

(3) Subjects were requested to rinse with 10 ml of sterile phosphate-buffered saline (PBS) for 1 min and to return the contents to a sterile container.

Laboratory analysis

All samples were collected between 09.00 and 12.00 h and processed within 1–2 h. ABO blood group was determined by slide agglutination with monoclonal anti-A and anti-B antibodies (Scottish National Blood Transfusion Service). Secretor status was determined by the presence of Lewis antigen on red blood cells by tube agglutination with monoclonal anti-$Lewis^a$ and anti-$Lewis^b$ antibodies (Scottish National Blood Transfusion Service). The haemagglutination inhibition method with boiled saliva [8] was used to confirm the Lewis antigen results for 159 individuals.

The swabs were incubated at 37 °C for 36–48 h, plated onto malt agar and incubated for a further 36–48 h.

The mouth rinse was concentrated by centrifugation at 2000 ***g*** and resuspended in 1 ml of PBS; 20 μl of the suspension were inoculated onto malt agar plates and incubated at 37 °C for 36–48 h. The number of colonies per sample were recorded.

Pure colonies were subcultured and identified with the API 20C AUX. Additionally all were identified by the conventional methods [9] of germ-tube production in horse serum, urease test and hyphae production on corn-meal agar following incubation at 28 °C for 48 h.

Statistical methods

All results were coded and a computerized database was set up to facilitate analysis using SPSSX. The relationship between prevalence of DS and other factors was tested by χ^2 (with Yates' correction) or Wilcoxon rank sum tests. Stepwise linear discriminant analysis was used to identify which combinations of factors best predicted the presence or absence of DS.

RESULTS

Oral conditions among the patients examined are summarized in Table 1. Of the 216 denture wearers, 76 (35%) had DS (Table 2). In 18% (14/76) the rinsing technique failed to isolate any yeasts. Similar results were obtained with the swab taken from the palate. The species most frequently isolated from the palate of patients with DS were *C. albicans* (33/76, 43%), followed by *Torulopsis glabrata* (6/76, 8%) and *C. tropicalis* (3/76, 4%). In 9%, the isolate could not be identified to a species level. Other species were isolated in 15% (11/76) of DS cases (Table 3).

Table 1. *Prevalence of oral conditions among diabetic individuals*

	Prevalence in total population screened	Prevalence among denture wearers
Gingivitis	114 (28%)	39 (42%)
Angular cheilitis	42 (10%)	25 (12%)
Denture stomatitis	76 (35%)	76 (35%)
Fissured tongue	25 (6%)	15 (7%)
Dry mouth	15 (3%)	13 (6%)
Geographic tongue	11 (3%)	7 (3%)
Median rhomboid glossitis	10 (2%)	7 (3%)
Hairy tongue	8 (2%)	5 (2%)
Glossitis	7 (2%)	5 (2·3%)
Leukoplakia	2 (0·5%)	2 (1%)

Table 2. *Prevalence of DS among patients with IDDM or NIDDM*

	+DS	−DS	Total
IDDM	32 (41%)	47 (59%)	79 (100%)
NIDDM	43 (32%)	91 (68%)	134 (100%)
	75	138	213

Missing, 3; $\chi^2 = 1{\cdot}20$; $P = 0{\cdot}27$.

Table 3. *Mycological profile of diabetic individuals (Palatal swab)*

Isolate	Denture wearers no. (%)	DS cases no. (%)
C. albicans	67 (31)	33 (43)
T. glabrata	13 (6)	6 (8)
T. beigelli	5 (2)	3 (4)
C. tropicalis	4 (2)	3 (4)
C. paratropicalis	3 (1)	2 (3)
S. cereviciae	2 (1)	0 (0)
C. stellatoidea	1 (0·5)	1 (1)
T. inconspicua	2 (1)	1 (1)
C. luscitaniae	1 (0·5)	0 (0)
C. humicola	1 (0·5)	0 (0·5)
C. pseudotropicalis	1 (0·5)	1 (1)
Unidentified	24 (11)	7 (9)
Missing	5 (2)	2 (3)
Other	4 (2)	3 (4)
No isolate	83 (38)	14 (18)
Total	216	76

Secretor status

There was a trend for non-secretors to be prone to DS (Table 4). Among individuals of blood group O with DS, non-secretors (13/27, 48%) appeared to be more prone to DS than secretors (19/68, 28%); $\chi^2 = 2{\cdot}69$, D.F. = 1, $P = 0{\cdot}1$. This was not seen among the 73 blood group A individuals. There were 10 (45%) A non-secretors compared with 15 (29%) A secretors with DS ($\chi^2 = 1{\cdot}1$, D.F. = 1, $P = 0{\cdot}3$).

Table 4. *Secretor status and prevalence of DS among patients with diabetes*

	Denture stomatitis			
	Present no. (%)	Absent no. (%)	χ^2	P
Patient category				
Total (n = 199)				
Secretor	45 (31)	100 (69)	2·56	0·11
Non-secretor	24 (44)	30 (56)		
IDDM				
Secretor	18 (40)	27 (60)	0·03	0·87
Non-secretor	13 (45)	16 (55)		
NIDDM				
Secretor	27 (27)	72 (73)	1·90	0·17
Non-secretor	11 (44)	14 (56)		

Table 5. *Relationship between DS and presence of denture in the mouth at night*

		Denture stomatitis			
Patient category	Presence of denture	Present No. (%)	Absent No. (%)	χ^2	P
Total*	Yes	45 (52)	42 (48)	23·2	< 0·000 01
	No	7 (12)	53 (88)		
IDDM	Yes	18 (49)	19 (51)	3·50	0·062
	No	3 (18)	14 (82)		
NIDDM	Yes	27 (54)	23 (46)	18·82	< 0·000 01
	No	4 (9)	39 (91)		

* Of a total of 216 denture wearers. 43 were recruited during the pilot study and were not questioned about their denture wearing habits. For 26 individuals this information was not recorded during the main study period.

Significant associations were found between DS and the following:

Presence of dentures in the mouth at night. Significantly more individuals who did not remove their dentures at night (45) had DS compared with those who removed their dentures at night (7) ($P < 0·000 01$). This was found particularly among NIDDM individuals (Table 5).

Number of colonies isolated by the mouth rinse technique. Subjects who did not have DS had significantly fewer colony forming units (median = 10 c.f.u./ml) than subjects with DS (median = 1850 c.f.u./ml, $P < 0·0001$). This was seen particularly for patients with NIDDM (median = 1500 c.f.u./ml with DS and 25 c.f.u./ml without DS, $P = 0·0003$). Those with IDDM did not show this relationship (median = 2400 c.f.u./ml with DS, 502·5 c.f.u./ml without DS, $P = 0·07$).

Random plasma glucose. Lower values for random plasma glucose were observed among the 132 patients without DS (median = 9·55 mmol/l) than those observed for the 72 patients with DS (median = 10·95 mmol/l, Mann–Whitney $P = 0·02$). This was seen particularly among individuals with NIDDM (median = 10·75 mmol/l with DS and 9·5 mmol/l without DS, $P = 0·04$). Individuals with IDDM

Table 6. *Stepwise discriminant analysis (Wilks)*

Sample	Cases predicted (%)	Variables isolated
IDDM ($n = 52$)	62·90%	Glycosuria ($P < 0·05$)
NIDDM ($n = 88$)	81·82%	Denture in at night ($P < 0·01$) No. of yeast colonies ($P < 0·01$) Non-secretion ($P < 0·01$)

did not show this relationship (median 11·250 mmol/l with DS and 10·15 mmol/l without DS, $P = 0·24$).

History of candidiasis. Among individuals with a history of superficial candidiasis 18/33 (55%) had DS compared with 58/182 (32%) with a negative history of candidiasis ($\chi^2 = 5·33$, D.F. = 1, $P = 0·02$). Separate analysis with respect to insulin dependency revealed a trend for individuals with a history of candidiasis to be more prone to DS: for IDDM individuals $\chi^2 = 1·2$, D.F. = 1, $P = 0·3$; for individuals with NIDDM, $\chi^2 = 3·26$, D.F. = 1, $P = 0·07$.

No significant association was found between DS and the following variables: sex; age; type of diabetes; control of diabetes (HBA_1); duration of diabetic state; ABO blood group; smoking; alcohol consumption; persistent glycosuria or albuminuria; denture fit, hygiene, occlusion, age or type (partial or full); presence of diabetic complications (neuropathy, nephropathy, retinopathy); contraceptive pill; antibiotics; and corticosteroids (systemic or topical).

In order to determine the relative contributions of variables in the development of DS, a stepwise discriminant analysis (SPSSX) was used. Initially, all variables were screened at an F value of 4 ($P < 0·05$). Analysis was then confined to the variables isolated as significant to increase the number of cases examined. Table 6 summarizes the contributory variables and the significance of their contribution in predicting infection. For patients with IDDM, the only variable identified by the analysis was persistent glycosuria ($P < 0·05$). For patients with NIDDM three factors were identified: denture present at night ($P < 0·01$), number of yeasts isolated ($P < 0·01$) and non-secretion ($P < 0·01$).

DISCUSSION

All forms of the oral conditions listed in Table 1 are more prevalent among the patients with dentures than those without dentures. Fissured tongue was encountered in 6% of the diabetic individuals. This figure is similar to that reported for 2478 dental patients [10]. The prevalence of geographic tongue and median rhomboid glossitis was slightly higher among diabetics than among dental and dermatology patients [11]. All the above conditions were much lower in prevalence than those reported for diabetic individuals by Farman who examined coloured South Africans [12]. These differences might be due to genetic and/or environmental factors between the predominantly Northern European population sampled here and that sampled by Farman. Dry mouth was a spontaneous complaint in only 3% of the samples compared to 34% reported by Sharon and colleagues [13].

The univariate analysis showed that prevalence of DS was similar among patients with IDDM or NIDDM. The prevalence of DS reported (35·2%) is within the range (24–60%) reported for non-diabetic individuals [3].

C. albicans was isolated from only 43% of the patients with DS. Isolation of *C. glabrata* (8%), and other yeasts (28%) from patients with DS indicate that, among diabetic individuals, species other than *C. albicans* are an important cause of disease.

Sex, denture trauma and hygiene [14–19], treatment with corticosteroids [21] and antibiotics [3], have variably been reported to be associated with DS among non-diabetic individuals. In this study, both the discriminant and univariate analyses did not reveal any association between these factors and DS.

Among patients with candida leukoplakia (CL), smoking is a significant factor in the pathogenesis of the lesion [20] which develops in sites away from the denture bearing area. In DS, smoking was not a factor in patients with either IDDM or NIDDM. This is consistent with the fact that the palate is protected from the effect of the smoke by the denture.

Control of diabetes as measured by glycosylated haemoglobin (HBA_1) was not associated with DS. Although this was unexpected at first sight, it is well established that blood glucose levels and salivary glucose levels among diabetic individuals do not correlate [13].

Discriminant analysis indicates that factors contributing to development of DS among patients with NIDDM are clearly different from those with IDDM. Patients with NIDDM who do not remove their denture at night, harbour a large number of yeasts and non-secretors of blood group antigens are particularly at risk of developing DS. In contrast, none of these factors influence the development of DS among individuals with IDDM. This is further supported by the results of the univariate analysis in which the P value for these factors among IDDM individuals are consistently higher than the P value among NIDDM individuals.

Although secretor status did not show a significant univariate association with DS among individuals with NIDDM, it was found to be significant in the multivariate analysis when adjusted for denture wearing habits and density of yeast colonization. This suggests that the univariate relationship might have been obscured by the other two contributory factors.

Persistent glycosuria was the only predictor of DS development among IDDM individuals. The presence of 11·1 mmol/l or more of glucose in the arterial blood results in the appearance of glucose in the urine. It was unexpected that neither HBA_1 nor random plasma glucose were implicated as both are more precise indicators of glucose availability than glycosuria. The possibility than glycosuria was a chance isolation cannot be dismissed especially since the significance of this variable was marginal. It appears that the discriminant analysis was not effective in identifying variables important to the development of DS among IDDM individuals.

Non-secretion of blood group antigens was a significant contributory factor among NIDDM but not among IDDM individuals. Non-secretion has been associated with carriage of yeasts among non-diabetic individuals [6] and patients with NIDDM [7]. This complements the results relating to the development of DS in these patients. It has been suggested that patients with IDDM are more

immunocompromized than NIDDM so that any protective effect of secretion of blood group antigens does not make a significant contribution to prevention of colonization or disease. A previous study [22] did not find an association between secretor status and DS among diabetics; however, the number of patients examined was much smaller, there was no differentiation between IDDM and NIDDM individuals and multivariate analysis was not applied to the data.

Identification of factors contributing to the development of DS among individuals with IDDM and NIDDM might have implications for treatment of this condition. Treatment of DS among patients with NIDDM might be similar to that in non-diabetic individuals [23], i.e. removal of denture, especially at night, which also reduces the density of colonization by yeasts. Among patients with IDDM, treatment of DS seems likely to depend on improvement of poor diabetic control reflected in persistent glycosuria.

ACKNOWLEDGEMENTS

Our thanks to Mrs M. Cole for typing the manuscript. This project was supported by the Wellcome Trust Foundation.

REFERENCES

1. Budtz-Jorgensen E. The significance of *C. albicans* in denture stomatitis. Scand J Dent Res 1974; **82**: 151–90.
2. Cawson RA. Denture sore mouth and angular cheilitis. Oral candidiasis in adults. Br Dental J 1963; **155**: 441–9.
3. Odds FC. Factors that predispose the host to candidiasis. In: Candida and candidasis: A review and bibliography. 2nd ed. Baillière Tindall, 1988: 93–114.
4. Blackwell CC. Genetic susceptibility to infectious agents. Proc Roy Coll Phys Edin 1989; **19**: 129–35.
5. Thom SM, Blackwell CC, MacCallum C, et al. Non-secretion of ABO blood group antigens and susceptibility to infection by *Candida* species. FEMS Microbiol Immunol 1989; **47**: 401–6.
6. Burford-Mason AP, Welsby JCP, Willoughby JMT. Oral carriage of *Candida albicans*, ABO blood group and secretor status in healthy individuals. J Med Veter Mycol 1988; **26**: 49–56.
7. Blackwell CC, Aly FZM, James VS, et al. Blood group, secretor status and oral carriage of yeasts among patients with diabetes mellitus. Diabetic Res 1989; **12**: 101–4.
8. Mollison PL. Blood transfusion in clinical medicine, 6th ed. Oxford: Blackwell, 1979; 414–82.
9. Milne LJR. Fungi. In: Practical medical microbiology. 13th ed. Edinburgh: Churchill Livingstone, 1988: 675–99.
10. Halperin V, Kolab S, Jefferis KR, Huddleston SO, Robinson HBG. The occurrence of Fordyce spots, benign migratory glossitis, median rhomboid glossitis and fissured tongue in 2478 dental patients. Oral Surg Oral Med Oral Pathol 1953; **6**: 1072–7.
11. McCarthy FP. A clinical and pathologic study of oral disease. JAMA 1941; **16**: 116.
12. Farman AG. Atrophic lesions of the tongue: a prevalence study among 175 diabetic patients. J Oral Pathol 1976; **5**: 255–64.
13. Sharon A, Ben-Ayreh H, Etzhak B, Yoram K, Szargel R, Gulman D. Salivary composition in diabetic patients. J Oral Med 1985; **40**: 23–6.
14. Davenport JC. The oral distribution of candida in denture stomatitis. Br Dent J 1970; **129**: 151–6.
15. Cawson RA. Chronic oral candidiasis, denture stomatitis and chronic hyperplastic candidiasis. In: Winner HI, Hurley eds. Symposium on candidal infections. Edinburgh: Churchill Livingstone, 1964; 138–53.

16. Budtz-Jörgensen E. An epidemiological study of yeasts in elderly denture wearers. Community Dent Oral Epidemiol 1975; **3**: 115–9.
17. Budtz-Jörgensen E. Bertram V. Denture stomatitis. I. The etiology in relation to trauma and infection. Acta Odontol Scand 1970; **28**: 71–92.
18. Neil DJ. Symposium on denture sore mouth I. An etiology review. Dent Practit 1965; **16**: 135–8.
19. Nyquist G. Denture sore mouth. Acta Odont Scand 1952; **10**: Suppl. 9.
20. Arendorf TM. Walker DM. Kingdom RJ. Roll JRS. Newcombe RG. Tobacco smoking and denture wearing in oral candidal leukoplakia. Br Dent J 1983; **155**: 340–3.
21. Vogt FC. The incidence of oral candidiasis with use of inhaled corticosteroid. Ann Allergy 1979; **43**: 205–10.
22. Lamey PJ. Secretor status, candidal carriage and candidal infection in patients with diabetes mellitus. J Oral Pathol 1988; **17**: 354–7.
23. Turrell AJW. Aetiology of inflamed upper denture-bearing tissues. Br Dent J 1966; **120**: 542–6.

radiographs are commonly inadequate for diagnosing conditions such as spondylitis, metastasis at the base of a pedicle, and listhesis,[4] and therefore, apparently normal routine radiographs should not be considered adequate if the clinical picture indicates important disease.

It is surprising that so few patients were fully examined and that so many were not examined before the radiographs were requested. Although patients may forget much of what their general practitioner tells them in a consultation, we consider that most will remember whether or not they have been examined. At this unit most patients will have had their examination performed within a few days of the request so that elapsed time does not become a major factor in accuracy of recall. We thus believe that our figures are a reasonable approximation to the truth. It can only be that most of the doctors in our sample currently make their decision to request radiographs based on the patient's history and that the examination findings are unlikely to alter this decision one way or the other. We hope that dissemination of the college guidelines will help to modify this decision making process.

Few of the general practitioners we contacted were aware of the college guidelines, and most expressed an interest in seeing them. Radiologists should strive to ensure that their local general practitioners are informed of the guidelines and should discuss their implications with them.

Despite the pessimism of some authors,[9] there is evidence that educating clinicians about radiology can reduce the number of unnecessary examinations,[20,21] and in view of the many patients referred to our department for lumbar spine radiography, we hope that widespread acceptance of the guidelines will result in optimal use of radiological services. We also believe that there is a need for a guided increase in public awareness regarding the radiation engendered in diagnostic radiology. Though we do not wish to cause unnecessary concern, the community as a whole benefit from a reduction in medical radiation that might follow reduced demand from patients to have radiographic examinations for painful but benign conditions.

1 National Radiological Protection Board. *Radiation exposure of the UK population – 1988 Review.* London: HMSO, 1989. NRPB-R227.
2 National Radiological Protection Board. *Living with radiation.* London: HMSO, 1989.
3 National Radiological Protection Board. *A national survey of doses to patients undergoing a selection of routine x ray examinations in English hospitals.* London: HMSO, 1986. NRPB-R200.
4 The Royal College of Radiologists. *Making the best use of a department of radiology: guidelines for doctors.* London: Royal College of Radiologists, 1989.
5 *The ionising radiations regulations, 1985.* London: HMSO, 1985.
6 International Commission on Radiation Protection. Statement from the 1987 meeting of the ICRP. *National Radiological Protection Board Radiological Protection Bulletin* 1987;86 suppl.
7 National Radiological Protection Board. *Patient dose reduction in diagnostic radiology.* London: HMSO, 1990.
8 Roberts CJ. Costs and benefits of skull radiography for head injury. A national study by the Royal College of Radiologists. *Lancet* 1981;ii:791-5.
9 Roberts CJ. The effective use of diagnostic radiology. *J R Coll Physicians Lond* 1984;18:62-5.
10 Roberts CJ. Annotation: towards the more effective use of diagnostic radiology: a review of the Royal College of Radiologists working party on the more effective use of diagnostic radiology, 1976 to 1986. *Clin Radiol* 1988;39:3-6.
11 Pearson R. Radiography in women of childbearing age. *BMJ* 1989;299:1175-6.
12 Edelstyn GA, Gillespie PT, Crebbel FS. Radiological demonstration of osseous metastases: experimental observations. *Clin Radiol* 1967;18:158-62.
13 Quintet RJ, Hadler NM. Diagnosis and treatment of back pain. *Semin Arthritis Rheum* 1979;8:261-87.
14 Waddell G. An approach to backache. *Br J Hosp Med* 1982;28:187-219.
15 Kelen GD, Noji EK, Doris PE. Guidelines for the use of lumbar spine radiography. *Ann Emerg Med* 1986;15:245-51.
16 Nachemson A. A critical look at conservative treatment for low back pain. In: Jayson M, ed. *The lumbar spine and back pain.* London: Sector, 1976:355-66.
17 South East Thames Regional Health Authority. Making the best of an imaging department: guidelines for doctors. London: SETRHA, 1990.
18 Burt WP. Radiology for back pain. *Clin Radiol* 1989;40:6-10.
19 Ryan M, Mooney G. *Research on medical practice variation: Where now?* Aberdeen: HERU, University of Aberdeen, 1991. (Available from HERU.)
20 Fowkes FGR, Evans RC, Williams LA, Gelbach SH, Cooke BRB, Roberts CJ. Implementation of guidelines for the use of skull radiographs in patients with head injuries. *Lancet* 1984;ii:795-6.
21 Fowkes FGR, Davies ER, Evans, KT, Green G, Hugh AE, Nolan DJ. Compliance with the Royal College of Radiologists guidelines on the use of preoperative chest radiographs. *Clin Radiol* 1987;38:45-8.

(Accepted 29 August 1991)

Association between secretor status and respiratory viral illness

M W Raza, C C Blackwell, P Molyneaux, V S James, M M Ogilvie, J M Inglis, D M Weir

...epartment of Medical
...icrobiology, University
...Edinburgh Medical
...chool, Edinburgh
...H8 9AG
...W Raza, MB, *postgraduate*
...*dent*
...C Blackwell, PHD, *senior*
...*turer*
...S James, FIMLS, *research*
...*hnician*
...M Ogilvie, MD, *senior*
...*turer*
...M Weir, FRCPED, *professor*

...gional Virus Laboratory,
...y Hospital, Edinburgh
...Molyneaux, MB, *senior*
...*ister*
...M Inglis, PHD, *director*

...rrespondence to:
...r Blackwell.

...BMJ 1991;303:815-8

Abstract

***Objective*—To determine whether non-secretion of blood group antigens is associated with respiratory virus diseases.**

***Design*—Study of secretor status in patients with respiratory virus diseases determined by an enzyme linked immunosorbent assay (ELISA) developed to identify Lewis (Le) blood group antigen phenotypes (Le^a non-secretor; Le^b secretor).**

***Subjects*—Patients aged 1 month to 90 years in hospital with respiratory virus diseases (584 nasal specimens).**

***Main outcome measures*—Criteria for validation of ELISA (congruence between results on ELISA testing of 1155 saliva samples from a previous study and previously established results on haemagglutination inhibition (HAI) testing, proportions of Le^a, Le^b, and Le^- phenotypes in 872 samples of nasal washings from a previous study compared with the normal population). Secretor status of patients determined by ELISA and viruses isolated.**

***Results*—Agreement between HAI and ELISA for 1155 saliva samples was 97%. Lewis antigens were detected by ELISA in 854 (97·9%) of nasal washings (Le^a 233 (26·7%), Le^b 621 (71·2%), and Le^- 18 (2·1%)) in proportions predicted for a northern European population. Secretors were significantly overrepresented among patients from whom influenza viruses A and B (55/64, 86%; $p<0·025$), rhinoviruses (63/72, 88%; $p<0·01$), respiratory syncytial virus (97/109, 89%; $p<0·0005$), and echoviruses (44/44, $p<0·0005$) had been isolated compared with the distribution of secretors in the local population.**

***Conclusion*—Secretion of blood group antigens is associated with respiratory virus diseases.**

Introduction

Susceptibility to a variety of bacterial and superficial fungal infections is associated with the genetically controlled inability of individual subjects to secrete the water soluble form of the ABO blood group antigens into body fluids (non-secretion).[1-7] Non-secretors are also significantly overrepresented among patients with some autoimmune diseases for which infectious triggers have been proposed.[8-12] Although studies of associations between ABO blood groups and susceptibility to natural or experimental viral infections have been reported,[13-15] there are no published studies of secretor status and viral infection. In this study we tested the hypothesis that non-secretors might also be at increased risk of viral illnesses.

Because the quantities of material available from

agreed with the results of the ELISA for 119 (96%) of those tested. The ELISA correctly identified the Lewis antigen present in nasal secretions of all 26 laboratory staff. Among the 872 nasal washings obtained from the Common Cold Unit, Lewis antigens were detected in 854 (97·9%): Le^a antigen in 233 (26·7%); Le^b antigen in 621 (71·2%); and no Lewis antigen in 18 (2·1%). This finding does not differ from the distribution of those phenotypes in most northern European populations.[8]

LEWIS PHENOTYPES OF LOCAL POPULATION

Among 363 blood specimens from the antenatal clinic in which the Lewis antigens were detected, 28% were Le^a antigen positive only and 72% were Le^b antigen positive (tabe II). These results were not significantly different from the proportions of non-secretors (26·6%) and secretors (73·4%) determined in a previous study by haemagglutination inhibition tests of 334 samples of saliva from local blood donors.[1]

LEWIS PHENOTYPE OF PATIENTS WITH VIRAL ILLNESSES

In 192 of the 584 (33%) patients' specimens examined by ELISA not enough Lewis antigen was detected for definite classification: 81 showed borderline readings, and in 111 no antigen was detectable. The results obtained with these specimens most probably reflect degradation of the antigens due to delays in their transportation to the laboratory. No virus was isolated from 38 (20%) of these 192 specimens. Among the 392 specimens in which either or both Lewis antigens were definitely detected, no virus was isolated from 36 (9%) ($\chi^2=12·17$, $p<0·0005$). The proportion of unclassifiable specimens did not vary significantly with respect to isolation of any particular virus. As the specimens giving negative and borderline results for Lewis antigens could not be classified as being from secretors or non-secretors they were eliminated from further analysis.

TABLE II—*Lewis phenotype and secretor status of patients and controls and results of virus culture*

Source of specimen	Virus isolated	Le^{a-b-} (non-secretors) No (%)	Le^{a-b-}/Le^{a-b-} (secretors) No (%)	χ^2	p Value	Odds ratio* (95% confidence interval)
Antenatal clinic controls		103 (28)	260 (72)			
Regional virus laboratory:						
	Influenza	9 (14)	55 (86)	5·74	<0·025	2·42 (1·13 to 5·77)
	A	7 (14)	44 (86)	4·92	<0·05	2·49 (1·06 to 6·76)
	B	2 (15)	11 (85)	0·51	>0·05	2·18 (0·46 to 20·53)
	Parainfluenza	17 (25)	50 (75)	0·13	>0·05	1·17 (0·63 to 2·26)
	Respiratory syncytial virus	12 (11)	97 (89)	12·77	<0·0005	3·20 (1·66 to 6·67)
	Rhinovirus	9 (12)	63 (88)	7·11	<0·01	2·77 (1·31 to 6·57)
	Echovirus	0	44 (100)	15·25	<0·0005	∞ (4·41 to ∞)
	None	9 (25)	27 (75)	0·06	>0·05	1·19 (0·52 to 2·97)

*Odds ratio compared with local controls.

Table II compares the isolation of viruses from non-secretors and secretors. Compared with the local population, there was a significantly higher proportion of secretors among subjects from whom the following viruses were isolated: influenza A virus ($p<0·05$), rhinovirus ($p<0·01$), respiratory syncytial virus ($p<0·0005$), and echoviruses ($p<0·0005$). Although 11 of 13 specimens containing influenza B virus were from secretors, the numbers were too small to be significant. This pattern was not observed for the 67 specimens from which parainfluenza virus was isolated or the 36 from which no virus was isolated. In these two groups of specimens the proportions that were Le^b antigen positive (secretors) and Le^a antigen positive (non-secretors) were similar to those of the local population.

Discussion

Determination of Lewis phenotype is a good control for haemagglutination inhibition assays for ABO antigens which have been the standard method for determining secretor status. Agreement between the Lewis phenotypes and results of the haemagglutination inhibition assay for 1089 saliva specimens was 97%. "False secretors," of Le^a phenotype but secretors by haemagglutination inhibition, were the predominant mismatched pairs (27/31, 87%). Results of a previous study indicate that these are most likely the result of contamination of saliva by blood owing to poor oral hygiene or periodontal disease among these subjects.[17] Dilution of small samples to provide enough material for the haemagglutination inhibition test is probably the source of the small proportion (0·004%) of "false non-secretors," who are of Le^b phenotype but non-secretors by haemagglutination inhibition. The ELISA method eliminates the problem of contamination of non-secretor saliva by red blood cells and it can be carried out on smaller volumes than those needed for haemagglutination inhibition. The method also detected Lewis antigens in 854 (98%) of the 872 specimens from the Common Cold Unit, indicating that the method can be used to detect these antigens in diluted nasal secretions.

The nasal washings from volunteers at the Common Cold Unit were originally collected for determining secretory antibody titres and were frozen soon after collection, which would preserve the Lewis antigens; analysis of the results found the expected proportion (2%) of specimens negative for Lewis antigen. The high proportion of specimens from patients in hospital with viral illness for which borderline readings were obtained or in which no Lewis antigen was detected might be due, in part, to collection techniques and time taken for transportation and processing the specimens. Blood group antigens cannot be reliably detected in saliva kept overnight at room temperature. There was a significantly higher proportion of unclassifiable specimens from which no virus was isolated (20%) compared with the proportion of specimens in which Lewis antigens were definitely detected but from which no virus was isolated (9%) ($p<0·0005$). The proportion of unclassifiable specimens was not greater among the very young age groups (<24 months), from whom nasopharyngeal secretions are usually obtained. Although the Lewis antigens were correctly identified in all the samples of transport medium inoculated with nasal swabs obtained from 26 laboratory staff, secretions provide a larger quantity of material for isolating virus and detecting Lewis antigen.

Previous studies found non-secretion to be associated with various bacterial diseases and superficial yeast infections and with carriage of some pathogenic bacteria or yeasts.[19-21] These findings contrast with our present finding in which secretors were overrepresented among those patients with significant symptoms of respiratory illness and from whom influenza A virus, rhinovirus, or respiratory syncytial virus were isolated. Secretors were also significantly overrepresented among those from whom echoviruses were isolated; these patients, however, had various illnesses including meningitis, fever, and vomiting. An increase in the proportion of secretors was not associated with isolation of parainfluenza virus or with the group of individual subjects from whom no virus was isolated.

This is the first report of associations between secretion of blood group antigens and infectious diseases. There is evidence that the Le^a antigen present in greater amounts on epithelial surfaces of non-secretors might be one of the receptors for *Candida* species,[22] and studies in progress indicate that this might be true for *Neisseria meningitidis*. The hypothesis

that antigens present only in secretors (Le^b) or in higher quantities in secretors (H type 1 in addition to H type 2)[7] might act as one of the receptors for some viruses is under investigation.

This work is supported by grants from the Scottish Cot Death Trust, TENOVUS-Scotland, and the North East Scotland Meningitis Appeal. We thank Dr R H Fraser and Dr G Inglis from the Glasgow and West of Scotland Blood Transfusion Service for helpful discussions and for the monoclonal antibodies used in this study, Dr D A J Tyrell and Mrs C Brown of the MRC Common Cold Unit for supplying specimens of nasal washings, Dr R A Elton for statistical advice, and Mrs M K Cole for preparing the manuscript. MWR has a grant from the Overseas Research Student Awards Scheme.

1 Haverkorn MJ, Goslings WRO. Streptococci, ABO blood groups and secretor status. *Am J Hum Genet* 1969;21:360-75.
2 Chaudhuri A, Das Adhikary CR. Possible role of blood-group secretory substances in the aetiology of cholera. *Trans R Soc Trop Med Hyg* 1978;72:664-5.
3 Kinane DF, Blackwell CC, Brettle RP, Weir DM, Winstanley FP, Elton RA. ABO blood group, secretor state, and susceptibility to recurrent urinary tract infection. *BMJ* 1982;285:7-9.
4 Blackwell CC, Jonsdottir K, Hanson M, Todd WTA, Chaudhuri AKR, Mathew B, *et al*. Non-secretion of ABO blood group antigens predisposing to infection by Neisseria meningitidis and Streptococcus pneumoniae. *Lancet* 1986;ii:284-5.
5 Blackwell CC, Jonsdottir K, Hanson MF, Weir DM. Non-secretion of ABO antigens predisposing to infection by Haemophilus influenzae. *Lancet* 1986;ii:687.
6 Blackwell CC, May SJ, Brettle RP, MacCallum CJ, Weir DM. Secretor state and immunoglobulin levels among women with recurrent urinary tract infections. *J Clin Lab Immunol* 1987;22:133-7.
7 Thom SM, Blackwell CC, MacCallum CJ, Weir DM, Kinane DF, Wray D. Non-secretion of ABO blood group antigens and susceptibility to infection by Candida species. *FEMS Microbiol Immunol* 1989;7:401-6.
8 Andersen J, Lauritzen E. Blood groups and diabetes mellitus. *Diabetes* 1960;9:29-4.
9 Blackwell CC, James VS, Weir DM, Gemmell JD, Patrick AW, Collier A, *et al*. Secretor status of patients with insulin dependent or non-insulin dependent diabetes mellitus. *BMJ* 1987;295:1024-5.
10 Collier A, Patrick AW, Toft AD, Blackwell CC, James VS, Weir DM. Increased prevalence of non-secretors in patients with Graves' disease: evidence for an infective aetiology. *BMJ* 1988;296:1162.
11 Shinebaum R, Blackwell CC, Forster PJG, Hurst NP, Weir DM, Niuki G. Non-secretion of ABO blood group antigens as host susceptibility factor in the spondylarthropathies. *BMJ* 1987;294:208-10.
12 Blackwell CC. Genetic susceptibility to infectious agents. *Proceedings of the Royal College of Physicians of Edinburgh* 1989;19:129-35.
13 Mackenzie JS, Fummell PJ. The effect of ABO blood groups on the incidence of epidemic influenza and on the response to live attenuated and detergent split influenza vaccines. *Journal of Hygiene* 1978;80:21-30.
14 McDonald JC, Zuckerman AJ. ABO blood groups and acute respiratory virus disease. *BMJ* 1962;ii:89-90.
15 Potter CW. Haemagglutination inhibition antibody to various influenza viruses and adenoviruses in individuals of blood groups A and O. *Journal of Hygiene* 1969;67:67-74.
16 Mollison PL. *Blood transfusion in clinical medicine*. 6th ed. Oxford: Blackwell, 1979:414-82.
17 Blackwell CC, Weir DM, James VS, Cartwright KAV, Stuart JM, Jones DM. The Stonehouse study: secretor status and carriage of Neisseria meningitidis and Neisseria lactamica. *Epidemiol Infect* 1989;102:1-10.
18 Mourant AE, Kopec AC, Domaniewska-Sobczak K. *The distribution of the human blood groups and other polymorphisms*. London: Oxford University Press, 1976.
19 Burford-Mason AP, Welsby JCP, Willoughby JMT. Oral carriage of Candida albicans, ABO blood group and secretor status in healthy individuals. *J Med Vet Mycol* 1988;26:49-56.
20 Blackwell CC, Aly FZM, James VS, Weir DM, Collier A, Patrick AW, *et al*. Blood group, secretor status and oral carriage of yeasts among patients with diabetes mellitus. *Diabet Res* 1989;12:101-4.
21 Blackwell CC, Weir DM, James VS, Todd WTA, Banatvala N, Chaudhuri AKR, *et al*. Secretor status, smoking and carriage of Neisseria meningitidis. *Epidemiol Infect* 1990;104:203-9.
22 May SJ, Blackwell CC, Weir DM. Lewis[a] blood group antigen, a receptor for Candida species. *FEMS Microbiol Immunol* 1989;47:407-10.
23 Rahat A, Stewart J, Blackwell CC, Weir DM. Semi-quantative determination of H type 1 and type 2 antigens on buccal epithelial cells and in saliva of secretors and non-secretors. *Vox Sang* 1990;57:101-5.

(*Accepted 17 July 1991*)

Mode of delivery after one caesarean section: audit of current practice in a health region

Catherine M Paterson, Nigel J St G Saunders

Abstract

***Objective*—To audit the subsequent obstetric management of women who had had one previous baby delivered by caesarean section.**

***Design*—Retrospective analysis of a regional obstetric database.**

***Setting*—Data derived from the 17 obstetric units in North West Thames region.**

***Subjects*—1059 women who delivered a singleton fetus of at least 37 weeks' gestation with a cephalic presentation in 1988 who had a history of one previous caesarean section and no other deliveries.**

***Main outcome measures*—Mode of delivery, postnatal morbidity, and duration of hospital stay.**

***Results*—395 (37%) women were delivered by elective repeat caesarean section and 664 (63%) were allowed a trial of labour. Maternal height and birth weight of the previous infant differed significantly between those who were and those who were not allowed to labour. 471 (71%) of those allowed to labour achieved a vaginal delivery. In individual units there was no significant correlation between the proportion of patients allowed to labour and the rate of the successful trial of labour. There was a trend towards greater success rates in units that allowed a longer duration of labour ($p<0.05$) and units with greater use of oxytocin for augmentation of labour (not significant). Both elective and intrapartum caesarean section was associated with a significantly higher rate of postnatal infection than vaginal delivery (14·7% and 16·0% *v* 3·4%).**

***Conclusions*—In patients with a history of caesarean section there is no evidence that the likelihood of successful vaginal delivery after trial of labour is modified by the proportion of such patients allowed the option of attempted vaginal delivery. Until selection criteria of adequate prognostic value can be identified a more liberal approach to allowing women a trial of labour seems justified.**

Introduction

The rising incidence of caesarean birth in Britain and elsewhere is a cause for concern both in terms of the associated increase in clinical and social morbidity for the mother and increased cost to the health service. Repeat caesarean section makes a major contribution to the overall rate of caesarean section. One strategy for reducing the rate of caesarean birth, therefore, is to allow women with a history of lower segment caesarean section the option of a trial of labour in their next pregnancy unless there are specific contraindications.

Many studies attest to the safety of a properly conducted trial of labour in women who have previously delivered by caesarean section, and successful vaginal delivery can be expected in around two thirds of such cases. In a comprehensive review Lavin *et al* concluded that a properly managed trial was associated with an acceptably low incidence of scar dehiscence and perinatal mortality.[1] Furthermore, no maternal deaths were identified. This is in contrast to the recognised contribution of repeat elective caesarean section to maternal mortality.[2]

Factors known to influence the outcome of a trial of

cademic Department of
bstetrics and
ynaecology, Imperial
ollege of Science,
echnology and Medicine,
Mary's Hospital Medical
hool, London W2 1PG
therine M Paterson,
COG, *research fellow*
igel J St G Saunders, MD,
nior lecturer

rrespondence to:
Paterson.

1 1991;303:818-21

Thrombocytosis and ischaemic complications in giant cell arteritis

Jacques De Keyser, Nina De Klippel, Guy Ebinger

Department of Neurology, Academisch Ziekenhuis, Vrije Universiteit Brussel, B 1090 Brussels, Belgium
Jacques De Keyser, PHD, *neurologist*
Nina De Klippel, MD, *assistant*
Guy Ebinger, PHD, *head of department*

Correspondence to:
Dr De Keyser.

BMJ 1991;303:825

Platelet counts may be raised in giant cell arteritis [1] but the clinical relevance of this has not been examined. We have investigated the possibility of an association between thrombocytosis and ocular or cerebral ischaemic events in giant cell arteritis.

Patients, methods, and results

We reviewed the medical records of patients with a diagnosis of giant cell arteritis who were admitted to the University Hospital of the Vrije Universiteit Brussel between 1984 and 1989. The diagnosis was based on clinical features, laboratory data (raised erythrocyte sedimentation rate), biopsy findings in temporal arteries, good response to corticosteroid treatment, and subsequent course. The study population consisted of 56 patients (31 women, 25 men) aged 60-89 years (mean 75·6 (SD 6·3)). Platelet count was determined as routine with an automated counter (normal range 150-400 × 10⁹/l) before corticosteroid treatment.

Patients were assigned to one of two groups based on the presence or absence of transient or permanent visual loss, transient cerebral ischaemic attack, or stroke. Age and sex distributions of the groups were similar (table). Eighteen patients (32%) had a history of ischaemic complications. Transient or permanent visual loss occurred in 13, transient cerebral ischaemic attacks in seven (all in the vertebrobasilar system), and stroke in two (one patient died from a brain stem infarction and the other had an infarction in the territory of the middle cerebral artery).

In the whole study population thrombocytosis (platelet count >400 × 10⁹/l) was found in 21 patients (37·5%). Linear regression analysis showed no correlation between platelet count and erythrocyte sedimentation rate ($r=0·06$) or haemoglobin concentration ($r=0·15$). There was no relation between thrombocytosis and positive biopsy findings in temporal arteries. In 13 patients with thrombocytosis platelet counts were remeasured two to three weeks after corticosteroid treatment had been started; values had returned to normal in all.

The group with ischaemic complications had a significantly higher prevalence of thrombocytosis ($p<0·01$) and a higher median platelet count ($p<0·001$) than the group without ischaemic complications (table).

Findings in 56 patients with giant cell arteritis

	Patients with ocular or cerebral ischaemic events (n=18)	Patients with no ocular or cerebral ischaemic events (n=38)
Median age (years) (SD)	76 (5)	75 (7)
No (%) of women	11 (61)	20 (53)
No (%) with thrombocytosis (platelet count >400 × 10⁹/l)	11 (61)**	10 (26)
Median platelet count (× 10⁹/l) (SD) [range]	475 (125) [309-710]***	338 (96) [163-597]
Median erythrocyte sedimentation rate (mm in first hour) (SD) [range]†	95·6 (31·4) [22-138]	91·3 (22·9) [38-134]
Median haemoglobin concentration (g/l) (SD) [range]	109 (22) [77-149]	114 (18) [77-151]

*p<0·01 (χ^2 test). ***p<0·001 (Mann-Whitney U test). †Westergren.

Comment

Thrombocytosis in giant cell arteritis results from an increased production of platelets.[2] Our results show an association between thrombocytosis and the occurrence of ocular and cerebral ischaemic complications in giant cell arteritis.

Reactive thrombocytosis has been detected in various other inflammatory diseases, such as rheumatoid arthritis, ankylosing spondylitis, and inflammatory bowel disease.[3] The exact underlying mechanism responsible for the increase in platelet production is unknown. A first possible explanation for our findings is that thrombocytosis in giant cell arteritis reflects a more severe degree of vasculitis. We found no correlation between platelet count and erythrocyte sedimentation rate.

A second possibility is that thrombocytosis might contribute to the ischaemic complications in giant cell arteritis. However, there have been no convincing reports that ischaemic manifestations occur as a direct consequence of reactive thrombocytosis in other disorders, at least not with the rather moderate increases in platelet count detected in our patients. The lumen of arteries affected by giant cell arteritis is usually reduced by severe thickening of the intima, and thrombosis is often found at the site of active inflammation.[1,2,4,5] Hence we cannot exclude the possibility that in some patients with giant cell arteritis thrombocytosis may be pathologically significant in narrowed inflamed arteries.

1 Calamia KT, Hunder GG. Clinical manifestations of giant-cell (temporal) arteritis. *Clin Rheum Dis* 1980;6:389-403.
2 Bengtsson B-A, Malmvall B-E. Giant cell arteritis. *Acta Med Scand* 1982;suppl No 658:1-102.
3 Selroos O. Thrombocytosis. *Acta Med Scand* 1973;193:431-6.
4 Goodman B. Temporal arteritis. *Am J Med* 1979;67:839-52.
5 Reich KA, Giansiracusa DF, Strongwater SL. Neurologic manifestations of giant-cell arteritis. *Am J Med* 1990;89:67-72.

(Accepted 11 June 1991)

Secretor status and heterosexual transmission of HIV

C C Blackwell, V S James, S Davidson, R Wyld, R P Brettle, R J Robertson, D M Weir

Correspondence to:
Dr Blackwell.

BMJ 1991;303:825-6

In contrast to previous studies on bacterial diseases and superficial fungal infections, in which non-secretors of the ABO blood group antigens were significantly overrepresented,[1,2] we found a higher proportion of secretors among patients with some viral illnesses.[1] Previously, we predicted that among patients who acquired HIV through intravenous drug use or anal intercourse the proportions of secretors and non-secretors would reflect that of the local population as these routes of transmission bypass normal mucosal defences.[1,2]

We examined the hypothesis that among subjects exposed to HIV through heterosexual activities a significantly higher proportion of secretors would become infected with the virus.

Subjects, methods, and results

We studied 219 subjects: 151 (99 men and 52 women) who acquired HIV through drug use; 14 homosexual or bisexual men; and 54 (15 men and 39 women) whose only risk factor when they entered the study was heterosexual intercourse with a partner infected with HIV.

epartment of Medical
Microbiology, University
f Edinburgh Medical
chool, Edinburgh
H8 9AG
C Blackwell, PHD, *senior
cturer*
S James, FIMLS, *research
chnician*
M Weir, FRCPE, *professor*

fectious Diseases Unit,
ity Hospital, Edinburgh
Davidson, SCM, *research
rse*
Wyld, SRN, *research nurse*
P Brettle, FRCP, *consultant*

luirhouse Medical Group,
dinburgh
J Robertson, FRCP, *general
actitioner*

Secretor status was determined from specimens of saliva by an enzyme linked immunosorbent assay (ELISA) for Lewis (Le) blood group antigens.[1] The proportions of secretors and non-secretors among participants in the study were compared with those of 363 women attending antenatal clinics (secretors 72%, non-secretors 28%).[1] The χ^2 test incorporating Yates's correction factor was used to analyse the results. Odds ratios and 95% confidence intervals were calculated by the exact method.

There were 148 (68%) secretors and 71 (32%) non-secretors in the study. This did not differ significantly from the proportions of secretors (72%) and non-secretors (28%) in the local population.[1] The proportions of secretors and non-secretors were not significantly different among the patients with HIV who had acquired the virus by drug use or homosexual or bisexual activities (table).

Among the 54 participants at risk through heterosexual intercourse, 26 were positive for the virus and 28 remained uninfected. The proportions of secretors (70%) and non-secretors (30%) in this group did not differ from those in the other groups of patients with HIV or in the local population. In the 26 participants who had acquired the virus 23 (88%) were secretors and three (12%) were non-secretors, and in the 28 who remained uninfected 15 (54%) were secretors and 13 (45%) were non-secretors ($\chi^2=6{\cdot}29$, $p<0{\cdot}025$).

Comment

These results agreed with the original predictions. The proportions of secretors and non-secretors were not different among subjects who had acquired the virus by intravenous drug use or by anal intercourse, routes which circumvent mucosal defences.[1,2]

In the accompanying paper (p 815)[3] we found a significantly higher proportion of secretors among patients with symptoms of viral disease from whom influenza A and B viruses, rhinoviruses, respiratory syncytial virus, or echoviruses were isolated. If it is assumed that these patients were not immune to the virus at the time of exposure, secretion of ABO blood group antigens seems to be associated with development of disease due to these particular viruses, which enter the host via mucosal surfaces. Among the subjects who seemed to have acquired HIV by heterosexual intercourse the proportion of secretors was higher than among those who acquired the virus by intravenous drug use or homosexual intercourse (88% *v* 67%) ($\chi^2=4{\cdot}068$; $p<0{\cdot}05$). The significantly lower proportion of secretors among the group exposed to the virus who remained uninfected suggests that non-secretors might be less susceptible to acquiring the virus through heterosexual intercourse.

Most work on transmission of HIV has been on factors associated with infected subjects rather than their sexual contact(s).[4] Secretor status is a characteristic easily determined by non-invasive inexpensive procedures. Inheritance and expression of secretor status are independent of sex, age, or environmental factors. Examination of secretor status might be valuable in analysing the apparent variability of other epidemiological factors associated with heterosexual transmission of HIV.

Elucidation of the interactions underlying the apparently increased susceptibility of secretors to acquiring HIV through mucous membranes might lead to new approaches in preventing infection through heterosexual intercourse.

cretor status and HIV category

tegory	Total No	Le[a] antigen positive (non-secretor) No (%)	Le[b] antigen positive (secretor) No (%)	Odds ratio* (95% confidence interval)
ntrols (antenatal clinic)	363	103 (28)	260 (72)	
V positive subjects:				
Total	191	58 (30)	133 (70)	0·91 (0·61 to 1·37)
Intravenous drug users	151	52 (34)	99 (66)	0·75 (0·49 to 1·16)
Homosexual or bisexual men	14	3 (21)	11 (79)	1·45 (0·37 to 8·26)
terosexual partners of HIV positive subjects:				
HIV positive	26	3 (12)	23 (88)	3·03 (0·89 to 16·10)
HIV negative	28	13 (46)	15 (54)	0·46 (0·20 to 1·09)

dds ratio compared with local controls.

This study was supported by grants from the Medical Research Council (SPG9006813) and from The Wellcome Trust (18046/1.5). We thank the medical and nursing staff of the infectious diseases unit for their help; the patients and their partners for their help and patience, without which the study would not have been possible; Dr R A Elton for statistical advice; Miss D A C Mackenzie for technical help; and Mrs M K Cole for preparing the manuscript.

1 Blackwell CC. The role of ABO blood groups and secretor status in host defences. *FEMS Microbiol Immunol* 1989;47:341-50.
2 Blackwell CC. Genetic susceptibility to infectious agents. *Proceedings of the Royal College of Physicians of Edinburgh* 1989;19:129-38.
3 Raza MW, Blackwell CC, Molyneaux P, James VS, Ogilvie MM, Inglis JM, *et al*. Secretor status and viral illnesses. *BMJ* 1991;303:815-8.
4 European Study Group. Risk factors for male to female transmission of HIV. *BMJ* 1989;298:411-5.

(*Accepted 17 July 1991*)

ONE HUNDRED YEARS AGO

A very valuable find of skeletons has been made in Egypt by Mr. Flinders Petrie, who has recently opened a number of tombs previously intact at Medum, belonging to the beginning of the fourth dynasty. This is the earliest known date of Egyptian remains, and that to which the Egyptians ascribe themselves. The skeletons are well preserved, but tender and friable. Some of them bear unmistakable evidence of rheumatic changes, and consequently indicate that at that very remote period man was subject to and suffered from this, as is now shown from its antiquity, venerable disease. No ornaments or objects of art, except occasionally some rough pottery or a wooden headrest, were found with these remains. The greater number were interred in a contracted position with the knees drawn up to the breast, even when the tomb was long enough to allow burial in the extended position, the body placed on the left side, wrapped in linen cloth, the head always to the north and the face to the east. A few, however, apparently the bodies of the highest class or race, were interred in the extended position along with vases of stone or pottery and headrests. At this period there is no trace of mummification. The essential difference in the mode of interment seems to point to difference of race, and it is probable that the contracted burials are those of the prehistoric race of Egypt, while the dynastic race were interred with the body extended. It is extremely interesting to find these contracted burials common at so early a date in Egypt, as a similar mode was adopted by the earliest inhabitants of Great Britain. Mr. Petrie has brought the skeletons to England, and deposited them at the College of Surgeons, where they are being treated so as to strengthen them and render them available for the anatomical investigation which Mr. Petrie intends to have made in order to determine, if possible, their ethnographical affinities. When this is done we shall doubtless also have a full description of any pathological condition which may be present.

(*British Medical Journal* 1891;ii:658)

INFECTION AND IMMUNITY, Nov. 1991, p. 4252–4254
0019-9567/91/114252-03$02.00/0

Vol. 59, No. 11

In Vitro Binding of *Helicobacter pylori* to Human Gastric Mucin

LEONIDAS S. TZOUVELEKIS,[1]* ANDREAS F. MENTIS,[1] ANTONIOS M. MAKRIS,[2] CHRISTOS SPILIADIS,[3] CAROLINE BLACKWELL,[1] AND DONALD M. WEIR[1]

Department of Bacteriology, Hellenic Pasteur Institute, 127 Vassilisis Sofias Avenue,[1] and Department of Internal Medicine, Hippocration Hospital,[2] 11521 Athens, and Department of Gastroenterology, Athens General Hospital, 11527 Athens,[3] Greece

Received 15 October 1990 Accepted 12 August 1991

The in vitro binding of four *Helicobacter pylori* strains to human gastric mucin was studied with an enzyme-linked immunosorbent assay. All four strains were found to bind to purified mucin. Neuraminidase treatment and nonspecific oxidation of mucin decreased bacterial adherence to the macromolecule. Mucin preparations were also found to inhibit attachment of *H. pylori* to HEp-2 monolayers.

The human gastrointestinal tract is covered with a continuous layer of mucus which act as a protective barrier for the underlying mucosa. The main component of mucus is a high-molecular-weight (>2 × 10^6) glycoprotein (mucin). Interactions between bacterial pathogens and mucins derived from humans and animal species have been reported in several studies (8, 11, 13, 15). Colonization of gastric mucosa by *Helicobacter pylori* is associated with chronic active antral gastritis and peptic ulceration (1, 2). The organism can be detected both attached to the gastric epithelial cells and free in the mucous layer (3, 6). In the present work, we studied some characteristics of *H. pylori* binding to human gastric mucin as well as the ability of mucin preparations to inhibit the adherence of the microorganism to HEp-2 cells.

Four *H. pylori* strains—W11, W82, W83, and W114—cultured from gastric-biopsy samples were used. The strains were characterized by colony and Gram stain morphology and production of urease, catalase, and oxidase. Mucosal scrapings of the fundus and pyloric antrum of stomachs obtained postmortem from two individuals with no history of gastrointestinal disease were used for the purification of gastric mucin. Mucin was purified as previously described (16) by equilibrium density gradient centrifugation in CsCl (twice) followed by filtration on a Sepharose 2B column (Pharmacia Fine Chemicals, Uppsala, Sweden). Purified mucin harvested from the void volume fractions of the column was dialyzed against distilled water, lyophilized, quantitated by dry weight, and stored at −70°C. Before use, mucin preparations were dissolved in phosphate-buffered saline (PBS; pH 7.4) at a final concentration of 400 μg ml. The absence of low-molecular-weight material was confirmed after the final preparation was run on a sodium dodecyl sulfate-polyacrylamide gel (4% stacking gel, 12% resolving gel) under nondenaturing conditions and stained with silver. In addition, 1-mg portions of purified mucin in 2.5 ml of PBS were either treated with 0.5 U of *Clostridium perfringens* neuraminidase type V (Sigma Chemical Co., St. Louis, Mo.) per ml for 15 min at 37°C or oxidized nonspecifically with sodium metaperiodate (5 mg ml) at 4°C for 30 min in the dark.

Bacterial binding to intact, neuraminidase-treated, and metaperiodate-oxidized mucins and the abilities of these three different mucin forms to inhibit bacterial adherence to HEp-2 cells were evaluated by an enzyme-linked immunosorbent assay (ELISA) utilizing anti-*H. pylori* rabbit serum prepared as previously described (12). A series of preliminary experiments with strain W11 were performed to determine whether ELISA readings corresponded to the number of bacteria on immobilized mucin and on HEp-2 cell monolayers. A good correlation between the ELISA readings and the numbers of bacteria counted microscopically was evident (Fig. 1). Also, in the same experiments, various parameters were probed in order to define the appropriate conditions for the experiments.

Wells of polystyrene microdilution plates (Nunc-Immunoplate Maxisorp; Nunc Inc., Roskilde, Denmark) were coated overnight at 4°C with 100 μl of untreated mucin. Mucin was omitted from control wells. After unbound material was washed off, the remaining sites in the mucin-coated and control wells were blocked with 4% (wt/vol) skim milk for 3 h at room temperature. Serial dilutions of bacteria in PBS were then added (100 μl per well). The initial suspension was adjusted to 10^9 CFU/ml. The plates were incubated for 2 h at 37°C under microaerophilic conditions. Unbound bacteria were removed by four washes with PBS containing Tween 20 (0.05% [vol/vol]). Anti-*H. pylori* immune serum (100 μl, 1/1,000 dilution) was then added. After incubation for 30 min at 37°C, the wells were washed with PBS-Tween 20 and incubated with 100 μl of a 1/2,000 dilution of swine anti-rabbit immunoglobulins conjugated to peroxidase (Dako Immunoglobulins, Glostrup, Denmark) for 30 min. After the washes, 100 μl of an *O*-phenylenediamine solution (0.4 mg ml) containing 0.4 μl of H_2O_2 (30% [vol/vol]) per ml was added as substrate and incubated at 37°C. After 10 min, the reaction was stopped with 100 μl of 1 N H_2SO_4, and the A_{490} was recorded in a MR5000 MicroELISA Autoreader (Dynatech Laboratories, Inc.).

Differences in bacterial binding to mucin after neuraminidase or metaperiodate treatment were estimated by microaerophilically incubating 5 × 10^7 PBS-suspended cells per strain with 20 μg of each mucin type for 30 min at 37°C. The total volume was 100 μl. In control wells, 20 μg of gelatin was added. The suspensions were then transferred to the wells of ELISA plates precoated with mucin and were incubated further for 90 min. The degree of binding was evaluated as described above.

The inhibitory effects of mucin preparations on *H. pylori* adherence to HEp-2 cells were examined as follows. HEp-2 cells were grown to confluence in 96-well tissue culture plates (Costar, Cambridge, Mass.). The monolayers were fixed with 0.25% glutaraldehyde for 10 min at 4°C and treated

* Corresponding author.

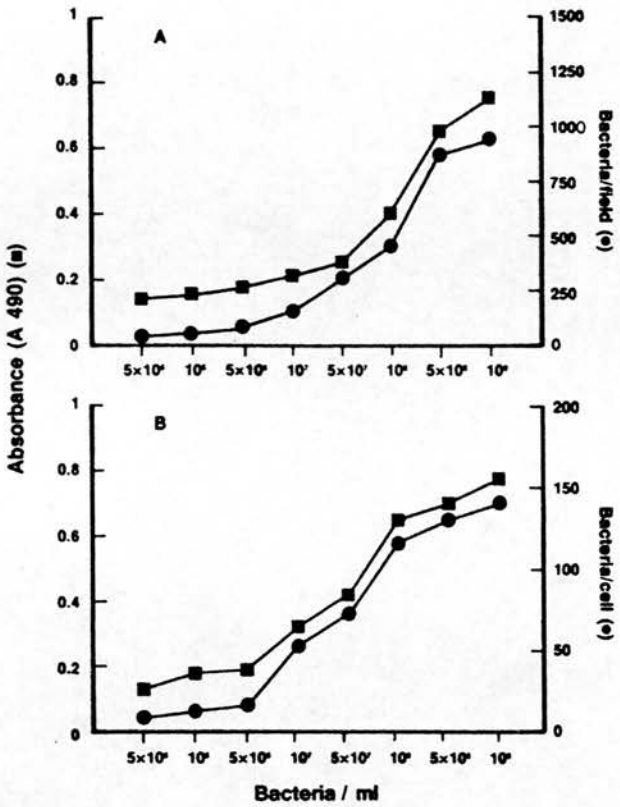


FIG. 1. Correlation of ELISA values with the number of bacteria bound on mucin (A) and attached to HEp-2 cells (B). ELISA was performed as described in the text. For direct microscopic counts, the same procedure was followed except that instead of *O*-phenylenediamine, diaminobenzidine was used as the substate. Bacteria were counted with an inverted microscope (magnification, ×400).

with 0.1% (wt/vol) gelatin for 1 h at room temperature. Fixation was necessary to retain monolayers intact throughout the multiple washes required for quantitation by ELISA. Twofold dilutions of each mucin form (50 μl) at an initial concentration of 400 μg/ml were placed in the wells. A constant amount of each *H. pylori* strain (approximately 3×10^7 CFU in 50 μl of PBS) was then added. After incubation at 37°C for 2 h under microaerophilic conditions, the plates were washed five times with PBS-Tween 20. To estimate the bacterial adherence, the ELISA procedure described above was followed. After color development was stopped, the

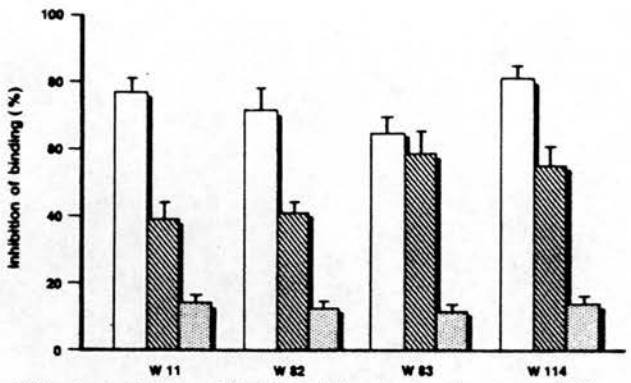


FIG. 2. Inhibition of binding of *H. pylori* strains to mucin immobilized in ELISA microplates by intact mucin (open bars), neuraminidase-treated mucin (hatched bars), and sodium metaperiodate-oxidized mucin (stippled bars). Data are presented as the mean values of five or more assays. Error bars show the standard deviations of mean values.

liquid content of each well was transferred to ELISA plates and the A_{490} was recorded. The results were analyzed by Student's *t* test.

All four *H. pylori* strains were found to bind to untreated mucin in progressively increasing numbers, reaching saturation when 5×10^7 CFU was added per well. At the saturation point, the mean values for ratios of binding to mucin/binding to blocking agent were 8.1 for strain W11, 7.6 for W82, 7.3 for W83, and 8.4 for W114. Binding curves were essentially the same for strains W11 and W82, showing maximum slopes at between 1×10^7 and 5×10^7 CFU added per well. Binding slopes were approximately the same between 1×10^6 and 5×10^7 CFU per well for both W83 and W114 strains. Binding ratios at the lower dilution used (10^6 CFU per well) were comparable for all strains tested, ranging from 3.2 to 3.8. Each ratio represents the average of 10 measurements not differing by more than 5%.

As shown in Fig. 2, incubation of bacteria with each of the mucin forms in the soluble state decreased bacterial binding to immobilized untreated mucin. The greatest inhibition was observed with homologous mucin. Mean inhibition was substantially less when neuraminidase-treated mucin was used, but the difference was not statistically significant in strain W83. Periodate-oxidized mucin affected binding only slightly, with inhibition percentages never exceeding 18%.

Experiments to assess inhibition of bacterial adherence to HEp-2 cells (Table 1) showed that in the presence of

TABLE 1. Percent inhibition of adherence of four *H. pylori* isolates to HEp-2 monolayers by three mucin types

Strain	Inhibition of bacterial adherence (% inhibition ± SD) at mucin concn of[a]:								
	200 μg/ml			100 μg/ml			50 μg/ml		
	UM	NM	PM	UM	NM	PM	UM	NM	PM
W11	75 ± 13	39 ± 3[b]	9 ± 3[b]	68 ± 5	16 ± 3[b]	5 ± 3[b]	21 ± 8	ND	ND
W82	88 ± 7	52 ± 10[b]	12 ± 1[b]	68 ± 10	24 ± 2[b]	ND	15 ± 4	11 ± 4[c]	ND
W83	62 ± 14	56 ± 4[b]	15 ± 3[b]	44 ± 4	30 ± 6[b]	8 ± 1[b]	9 ± 2	6 ± 1[c]	ND
W110	85 ± 8	55 ± 9[b]	12 ± 2[b]	80 ± 12	28 ± 7[b]	ND	16 ± 5	ND	ND

[a] Percent inhibition was calculated as follows: $[(A_{490}$ of control $- A_{490}$ in the presence of mucin) $\times 100]/A_{490}$ of control). Each value is the average of 10 assays. *P* values were obtained by comparing inhibition caused by untreated mucin with inhibition caused by each of the two forms of treated mucin. UM, untreated mucin; NM, neuraminidase-treated mucin; PM, periodate-oxidized mucin; ND, not detected.

[b] $P < 0.01$.

[c] $P < 0.05$.

untreated mucin, adherence of all *H. pylori* strains was markedly reduced. Increasing concentrations of mucin enhanced the degree of inhibition. Bacterial adherence could be inhibited with as little as 25 μg of mucin per ml. Below this concentration, no inhibition was detected. The inhibitory effect was significantly lower with neuraminidase-treated mucin for all but W83 strains. An even greater loss of inhibitory activity was observed after metaperiodate oxidation of mucin.

Studies on the adherence properties of *H. pylori* have shown it to possess a fibrillar hemagglutinin which recognizes sialic acid-containing structures on erythrocytes (5) and on mouse Y-1 adrenal cells (4). It has also been found that mucins of different origin, such as porcine gastric mucin, bovine submaxillary mucin (10), and human salivary mucin (9), are able to inhibit *H. pylori* hemagglutination activity. Additionally, in the case of human salivary mucins, removal of sialic acid resulted in a substantial decrease in their inhibitory effect. These observations might indicate that outer surface structures of *H. pylori* interact with sialic acid-terminated molecules present on gastric epithelial cells as well as with analogous structures present in soluble form in the gastric mucous layer.

The present study provides evidence that the organism is able to bind to human gastric mucin and that this binding might prevent adherence of the organism to epithelial cells. Sialic acids appear to be partly responsible for this interaction. However, nonspecific oxidation of the sugar moieties of mucin was required to further decrease binding. This indicates that carbohydrate structures other than sialic acids also contribute to the binding.

Although we cannot assign any particular role to the interaction between the microorganism and the gastric mucin, this interaction might protect against *H. pylori* by inhibiting attachment of the organism to the underlying gastric epithelial cells, thus assisting removal of *H. pylori* by the continuous flow of mucus. Alternatively, as has been suggested for other pathogens (7, 8), attachment of *H. pylori* to gastric mucin might facilitate dissemination of the organism in the stomach mucous layer and subsequent colonization of the underlying mucosa.

It is believed that the specific characteristics of mucins and invading microorganisms affect their abilities to interact. Human gastric mucin appears to be altered in patients with certain diseases such as gastritis or peptic ulceration (16). Additionally, as reported by Slomiany et al. (14), *H. pylori* can enzymatically degrade mucin, thus altering its physicochemical properties. Therefore, the detailed study of *H. pylori* interactions with several gastric mucin types may help explain the colonization and pathogenic processes.

REFERENCES

1. **Blaser, E. H.** 1987. Gastric *Campylobacter*-like organisms, gastritis and peptic ulcer disease. Gastroenterology **93**:371–383.
2. **Buck, G. E., W. K. Gourley, W. K. Lee, K. Subramanyam, J. M. Latimer, and A. R. DiNuzzo.** 1986. Relation of *Campylobacter pyloridis* to gastritis and peptic ulcer. J. Infect. Dis. **153**:664–669.
3. **Chen, X. G., P. Correa, J. Offerhaus, E. Rodriguez, F. Janney, E. Hoffman, J. Fox, F. Hunter, and S. Diavolitsis.** 1986. Ultrastructure of the gastric mucosa harboring *Campylobacter*-like organisms. Am. J. Clin. Pathol. **86**:575–582.
4. **Evans, D. G., D. J. Evans, Jr., and D. Y. Graham.** 1989. Receptor-mediated adherence of *Campylobacter pylori* to mouse Y-1 adrenal cell monolayers. Infect. Immun. **57**:2272–2278.
5. **Evans, D. G., D. J. Evans, Jr., J. J. Moulds, and D. Y. Graham.** 1988. *N*-Acetylneuraminyllactose-binding fibrillar hemagglutinin of *Campylobacter pylori*: a putative colonization factor antigen. Infect. Immun. **56**:2896–2906.
6. **Goodwin, C. S., J. A. Armstrong, and B. J. Marshall.** 1986. *Campylobacter pyloridis* gastritis and peptic ulceration. J. Clin. Pathol. **39**:353–365.
7. **Lee, A., J. L. O'Rourke, P. J. Barrington, and T. J. Trust.** 1986. Mucus colonization as a determinant of pathogenicity in intestinal infection by *Campylobacter jejuni*: a mouse cecal model. Infect. Immun. **51**:536–546.
8. **Mantle, M., L. Basaraba, S. C. Peacock, and D. G. Gall.** 1989. Binding of *Yersinia enterocolitica* to rabbit intestinal brush border membranes, mucus, and mucin. Infect. Immun. **57**:3292–3299.
9. **Mentis, A., L. Tzouvelekis, C. Spiliadis, C. C. Blackwell, and D. M. Weir.** 1990. Inhibition of *Helicobacter pylori* haemagglutination activity by human salivary mucins. FEMS Microbiol. Immunol. **64**:125–128.
10. **Nakazawa, T., M. Ishibashi, H. Konishi, T. Takemoto, M. Shigeeda, and T. Kochiyama.** 1989. Hemagglutination activity of *Campylobacter pylori*. Infect. Immun. **57**:989–991.
11. **Nelson, J. W., M. W. Tredgett, J. K. Sheehan, D. Thornton, D. Notman, and J. R. W. Govan.** 1990. Mucinophilic and chemotactic properties of *Pseudomonas aeruginosa* in relation to pulmonary colonization in cystic fibrosis. Infect. Immun. **58**:1489–1495.
12. **Perez-Perez, G. I., and M. J. Blaser.** 1987. Conservation and diversity of *Campylobacter pyloridis* major antigens. Infect. Immun. **55**:1256–1263.
13. **Ramphal, R., N. Houdret, L. Koo, G. Lamblin, and P. Roussel.** 1989. Differences in adhesion of *Pseudomonas aeruginosa* to mucin glycopeptides from sputa of patients with cystic fibrosis and chronic bronchitis. Infect. Immun. **57**:3066–3071.
14. **Slomiany, B. L., J. Bilski, J. Serosisk, V. L. N. Marty, H. Dworkin, K. VanHorn, J. Zielenski, and A. Slomiany.** 1987. *Campylobacter pyloridis* degrades mucin and undermines gastric mucosal integrity. Biochem. Biophys. Res. Commun. **14**:307–314.
15. **Wanke, C. A., S. Cronan, C. Goss, K. Chadee, and R. L. Guerrant.** 1990. Characterization of binding of *Escherichia coli* strains which are enteropathogens to small-bowel mucin. Infect. Immun. **58**:794–800.
16. **Younan, F., J. Pearson, A. Allen, and C. Venables.** 1982. Changes in the structure of the mucous gel on the mucosal surface of the stomach in association with peptic ulcer disease. Gastroenterology **82**:827–831.

Epidemiol. Infect. (1992), **108**, 441–448
Printed in Great Britain

Factors affecting carriage of *Neisseria meningitidis* among Greek military recruits

C. C. BLACKWELL[1], G. TZANAKAKI[2], J. KREMASTINOU[3], D. M. WEIR[1], N. VAKALIS[3], R. A. ELTON[4], A. MENTIS[2] AND N. FATOUROS[3]

[1] *Department of Medical Microbiology, The Medical School, University of Edinburgh, Edinburgh EH8 9AG*
[2] *National Meningitis Reference Laboratory, Hellenic Institute Pasteur, Athens, Greece*
[3] *Athens School of Public Health, Athens, Greece*
[4] *Department of Public Health Sciences, University of Edinburgh*

(*Accepted 10 November 1991*)

SUMMARY

Greek military recruits (993) were examined for carriage of meningococci during July 1990. Blood, saliva and throat swab specimens were obtained and each recruit answered a questionnaire providing information on age, education (a measure of socioeconomic level), place of residence, smoking habits and recent infections.

The overall carriage rate was 25% but differed between the two camps: 79/432 (18%) in Camp A and 168/561 (30%) in Camp B ($P < 0{\cdot}0005$). In Camp B, there were significantly higher proportions of recruits who were non-secretors ($P < 0{\cdot}0005$), and/or heavy smokers ($P < 0{\cdot}0005$). They were also younger (< 19 years old) ($P < 0{\cdot}001$), and on the whole had fewer years of education ($P < 0{\cdot}0005$). By univariate analysis, carriage was significantly associated with smoking. By multiple logistic regression analysis, carriage was associated with smoking ($P < 0{\cdot}001$), age ($P < 0{\cdot}01$) and the camp in which the recruits were based ($P < 0{\cdot}01$). Among recruits in Camp B, 15/38 (40%) of those with recent viral infections were carriers compared with 30% for the camp in general.

INTRODUCTION

Following the epidemic of meningitis during the 1960s, the incidence of meningococcal disease in Greece declined steadily until 1988. Since 1988, the number of cases reported to the Ministry of Health has increased: in the first 6 months of 1990, there were 102 cases compared with 87 for all of 1989 (Table 1). This apparent increase in disease due to *Neisseria meningitidis* prompted the present work as the last study of meningococcal carriage in a Greek population was carried out before elucidation of some of the genetic and environmental factors affecting carriage [1].

Epidemiological studies have shown that non-secretors, individuals incapable of secreting the glycoprotein form of their ABO blood group antigens, are significantly over-represented among patients with meningococcal disease [2, 3]

Table 1. *Incidence of meningococcal disease in Greece*

Year	No. cases	Deaths no. (%)
1968	1064	48 (4·5)
1973	765	39 (5·1)
1978	286	18 (6·3)
1983	102	9 (8·8)
1985	105	6 (5·7)
1986	88	7 (7·9)
1987	85	4 (4·7)
1988	76	2 (2·6)
1989	87	6 (6·9)
1990 (1st 6 months)	102	

and among carriers in a school population [4]. Prolonged outbreaks have occurred in countries such as Iceland and in areas of Britain [5, 6] where the proportion of non-secretors is significantly higher than the proportion (20–25%) predicted for European populations [7].

Environmental factors have also been suggested as playing a role in susceptibility to meningococcal disease [8, 9] or carriage of the bacteria. Smoking or passive exposure to cigarette smoke has been associated with isolation of *Neisseria meningitidis* from healthy asymptomatic adults and teenagers [4, 10]. Virus infections have been suggested as predisposing factors for susceptibility to meningococcal disease [11] and to carriage of potentially pathogenic bacteria [12].

In the study reported here we wished to determine: (1) if isolation of meningococci was associated with non-secretion in the populations examined; (2) if smoking was associated with carriage in a closed population; (3) if an upper respiratory tract or other infection might be a factor contributing to carriage of meningococci; (4) if there might be geographical regions in Greece in which there are higher proportions of non-secretors and if the prevalence of meningococcal disease was increased in these areas.

SUBJECTS AND METHODS

Greek military recruits (993) from two camps, one at Athens and another at Avlona, were examined for carriage of meningococci during the third week of July 1990. Ethical permission for the study was granted by the Ministry of Defence. An explanation of the purpose of the study and the specimens required was part of the questionnaire and each subject signed his consent to participate in the study. The recruits came from all regions of Greece except the Ionian islands, and had been in the camps from 3 to 5 days at the time of the study. Throat swabs, blood and saliva specimens were obtained. Throat swabbing was carried out by the same four members of the team on both occasions (C.C.B., G.T., A.M., D.M.W.). Blood was not obtained from a number of recruits who fainted because of the unusually high temperature (41 °C). Each recruit filled in a questionnaire providing information on age, occupation, education level, places of residence and birth, smoking habits, and recent infections. A member of the research team checked the form to ensure all categories had been completed and to answer any questions. This information

and results of laboratory examinations were coded for confidentiality and stored in a Database 3 plus data base. The information on the data base was recalled and checked with the original questionnaire.

ABO blood group was determined by slide agglutination with monoclonal anti-A and anti-B antibodies (Scottish National Blood Transfusion Service): Lewis blood group was determined by tube agglutination with monoclonal anti-Lewis^a (Le^a) and anti-Lewis^b antibodies (Scottish National Blood Transfusion Service). Non-secretors can express only Le^a, but secretors express Le^b. The blood specimens were centrifuged on the day of collection and the plasma stored at −20 °C.

Throat swabs were plated directly onto modified New York City medium [13] and incubated for 48 h. Colonies were examined for oxidase production. Gram stain and carbohydrate utilization.

Two-way frequency tables were analysed by χ^2 tests with Yates' correction in the case of 2×2 tables. Multiple logistical regression was used to examine the association between carriage rates and other factors adjusted for each other.

RESULTS

Isolation of N. meningitidis

N. meningitidis was isolated from 247 (25%) of the 993 recruits examined. The carriage rate differed significantly between the two camps. There were 79 isolates obtained from 432 recruits (18%) in the camp at Athens compared with 168 from the 561 (30%) at Avlona ($\chi^2 = 17{\cdot}54$: $P < 0{\cdot}0005$).

Characteristics of the two populations

Distribution of ABO and Lewis blood group phenotypes

There was no difference in the distribution of the ABO blood groups between the two camps. Among the recruits who expressed Lewis antigen, there was a significantly higher proportion of Le^a/non-secretors in the camp at Avlona, 140/534 (26%), compared with that in the camp at Athens, 60/390 (15%) ($\chi^2 = 14{\cdot}96$, $P < 0{\cdot}0005$).

Age and education

The ages of the two populations differed significantly. The median age was 19 for both camps: however, there was a higher proportion of recruits under 19 in the camp at Avlona (4%) than in that at Athens (1%) ($\chi^2 = 12{\cdot}96$, $P < 0{\cdot}001$).

There was also a significant difference in the level of education of the recruits in the two camps, particularly at the two extremes. Among the recruits at Avlona 14% had only 6 years of schooling compared with 7% among those at Athens: and, only 2% of the recruits at Avlona had attended university while 7% of those at Athens had done so ($\chi^2 = 30{\cdot}32$, df = 4, $P < 0{\cdot}0005$).

Smoking

There was no significant difference between the proportion of smokers in the Athens camp (62%) and in Avlona (68%): but there were significantly more heavy smokers (> 30 cigarettes a day) in Avlona ($\chi^2 = 27{\cdot}64$, df = 4, $P < 0{\cdot}0005$) (Table 2).

Table 2. *Cigarette consumption in the two recruit groups*

No. cigarettes/day	Athens ($n = 431$) No. (%)	Avlona ($n = 560$) No. (%)
0	166 (38)	182 (32)
1–10	53 (12)	63 (11)
11–20	127 (29)	133 (24)
21–30	62 (14)	96 (17)
> 30	23 (5)	86 (15)

Table 3. *Carriage rates and cigarette consumption*

No. cigarettes/day	Total	Carriers no. (%)	Non-carriers no. (%)
0	355	56 (16)	299 (84)
1–10	116	29 (25)	87 (75)
11–20	260	66 (25)	194 (75)
21–30	157	44 (28)	113 (72)
> 30	109	40 (37)	69 (63)

Prevalence of other infectious diseases

Only 59 of the recruits reported symptoms of upper respiratory tract infections (URTI) within the previous 2 weeks, 21 of the 432 at Athens (4·9%) and 38 of the 561 (6·7%) at Avlona.

Factors associated with carriage of meningococci

There was no difference in the proportions of carriers among the four ABO blood groups. The proportion of carriers among Le^a/non-secretors (22% at Athens, 32% at Avlona) was higher than that among Le^b/secretors (17% at Athens, 30% at Avlona), but the difference was not significant.

The proportion of carriers was significantly higher among smokers and was greatest among those smoking more than 30 cigarettes per day (Table 3) ($\chi^2 = 23{\cdot}19$, df = 4, $P < 0{\cdot}0005$). Although the proportion of carriers was significantly increased among smokers in both camps (Athens, $\chi^2 = 5{\cdot}42$, df = 1, $P < 0{\cdot}05$; Avlona $\chi^2 = 9{\cdot}05$, df = 1, $P < 0{\cdot}01$), when the data for carriers were analysed by χ^2 test, there was no significant difference between the two camps associated with numbers of cigarettes per day ($\chi^2 = 9{\cdot}482$, df = 4, $P > 0{\cdot}05$) (Table 4).

Meningococci were isolated from 17 (29%) of the 59 recruits who had symptoms of URTI, 2 of the 21 (9·5%) at Athens and 15 of the 38 (39·5%) at Avlona. Among the 15 at Avlona from whom meningococci were isolated, 6 (40%) were Le^a/non-secretors compared with 4 (20%) Le^a/non-secretors among the 20 with recent symptoms of URTI but from whom no meningococci were isolated. The numbers of recruits with symptoms of URTI were too small for statistical analysis.

Multiple logistic regression analysis identified three main factors which were significantly associated with carriage: smoking ($P < 0{\cdot}001$); age less than 19 years ($P < 0{\cdot}01$) and the camp in which the recruits were based ($P < 0{\cdot}01$). Various

Table 4. *Smoking patterns and carriage of* N. meningitidis *in the two camps*

No. cigarettes/ day	Athens (n = 431) Total No. (%)	Athens (n = 431) Carriers No. (%)	Avlona (n = 560) Total No. (%)	Avlona (n = 560) Carriers No. (%)
0	166 (38)	20 (27)	182 (32)	36 (22·5)
1–10	53 (12)	10 (13)	63 (11)	19 (12)
11–20	127 (29)	26 (35)	133 (24)	40 (25)
21–30	62 (14)	15 (20)	96 (17)	29 (18)
> 30	23 (5)	4 (15)	86 (15)	36 (22·5)
Total	431	75	560	160

combinations of categories were tested for difference in carriage rate when adjusted for these three factors but no other variables were found to be significant.

Regional variations in ABO and Lewis blood group antigens

There was no difference in the distribution of the ABO blood groups in the two major population centres, Athens and Eastern Macedonia. There was, however, a significantly higher proportion of Le^a/non-secretors, 54/183 (29·5%) among the recruits from Eastern Macedonia compared with those from Athens, 35/235 (15%) ($\chi^2 = 12{\cdot}25$, $P < 0{\cdot}0005$).

DISCUSSION

As expected, compared with carriage rates reported for civilian populations (5–8% for Greek secondary school children), there was a higher proportion of carriers (25%) among the recruits examined in this study. This figure is similar to that found for a British military establishment (23%) [14] but slightly lower than that found in the 1970s for Greek military recruits (33%) [1]. The significant difference in the proportion of carriers between the two camps, 18% at Athens compared with 30% at Avlona ($P < 0{\cdot}0005$), was not anticipated.

Smoking [4, 10], passive exposure to cigarette smoke [10], secretor status [4] and age [15] have all been reported to be associated with carriage of meningococci. The distribution of the ABO blood group phenotypes did not differ between the two camps and were similar to those we found in a previous study [16] and that reported by Hirzfeld and Hirzfeld in 1919 [17]. Several of the factors examined in the study varied between the two camps. Compared with the recruits at Athens, among those at Avlona where there was a significantly higher proportion of carriers of meningococci, there were significantly more heavy smokers, non-secretors, younger recruits (less than 19 years old), and recruits of lower socieconomic groups as assessed by years of education.

Smoking was the factor most strongly associated with carriage in both univariate and multivariate analyses. It has been suggested that the higher proportion of smokers and heavy smokers among military personnel [18] might contribute to the increased prevalence of meningococcal disease and the higher rates of carriage among military recruits. The proportions of smokers in the two camps, 62 and 68%, were higher than the 47% found in a previous study among Greek male patients referred for gastroscopy [15]. In Britain smoking is associated

with socioeconomic status: unskilled individuals are more likely to smoke than those in professional groups [19]. A similar pattern was observed among the recruits: those with fewer years of education were more likely to be smokers.

In a previous study of teachers and pupils in a Scottish secondary school following an outbreak of meningitis, we found an association between non-secretion and carriage of meningococci [4]. In the present study, in both camps, the proportions of non-secretors were increased among carriers; but, these differences were not significant compared with the secretors among carriers. Assessment of both host and parasite characteristics might elucidate the differences in association with secretor status. The majority (35·5%) of serotypable strains among the 121 isolates in the Scottish study were serotype 4; and 55% of the carriers of this serotype were non-secretors. Only 9 (1·8%) isolates in the present study were serotype 4, but 37·5% of the carriers were non-secretors. The proportion of non-secretors among carriers of serotype 14 isolates was 25 and 22% for the Scottish and Greek populations. A similar pattern was observed for non-serotypable isolates.

The multiple logistic regression analysis identified age (those less than 19 years of age) to be a significant variable ($P < 0{\cdot}01$). This agrees with the findings for carriers in the Stonehouse survey. The highest proportion of carriers was found in the 15–19 year age group [15].

There is evidence that viral infections can predispose individuals to carriage of potentially pathogenic bacteria [12]; and binding of streptococci and staphylococci to cell surfaces is enhanced in experimental models examining infection with influenza virus [20, 21]. The present study was carried out in July when there is a low prevalence of upper respiratory viral infections; however, of the 59 recruits with symptoms of virus infection, 29% were carriers. This was particularly apparent among recruits at Avlona where the carriage rate was 30% overall but nearly 40% among those with virus infections. In a recent study, a higher proportion of patients with meningococcal disease had a history of flu-like illness than age- and sex-matched controls; however, the differences were not statistically significant [22]. A second study of recruits was undertaken in January 1991 to assess carriage when viral infections are more prevalent; the analysis of this study is not complete at present.

In two areas of Britain where there have been prolonged outbreaks of meningococcal disease, the proportions of non-secretors have been reported to be higher than the 20–25% predicted for European populations [5, 6]. In this study we found a significantly higher proportion of recruits from Eastern Macedonia were non-secretors (29·5%) compared with the other major centre of population, Athens (15·3%). As there are anecdotal reports that meningococcal disease is more prevalent in Macedonia, the numbers of cases reported to the Ministry of Health from these two areas is now being closely monitored.

ACKNOWLEDGEMENTS

We are grateful to the Ministry of Defence for permission to carry out this study and to the officers and recruits for their participation. The study was carried out with the expert assistance of: G. Voloudaki, A. Tabaki, G. Boursinos, G. Mitrakos,

T. Garanis, N. Kikemeni, P. Konstandinopoulo and T. Priftis. We are also grateful to Mrs J. G. Blackwell for assistance with the data processing and Mrs M. K. Cole for preparation of the manuscript.

REFERENCES

1. Kalapothaki V, Vassilliadis P, Trichopoulos D, Kaklamani E, Sérié Ch. Caractéristiques séro-épidémiologiques chez les porteurs sains de meningocoques en Grèce. Annales Microbiol (Institute Pasteur) 1979; **130B**: 43–54.
2. Blackwell CC, Jónsdottir K, Hanson M, et al. Non-secretion of ABO blood group antigens predisposing to infection by *Neisseria meningitidis* and *Streptococcus pneumoniae*. Lancet 1986; ii: 284–5.
3. Blackwell CC, Jonsdóttir K, Mohammed I, Weir DM. Non-secretion of blood group antigens. A genetic factor predisposing to infection by *Neisseria meningitidis*. In: Poolman JT, Zanen HC, Meyer TF, et al, editors. Gonococci and meningococci. Dordrecht: Kluwer Academic Publishers, 1988: 633–6.
4. Blackwell CC, Weir DM, James VS, et al. Secretor status, smoking and carriage of *Neisseria meningitidis*. Epidemiol Infect 1990; **104**: 203–9.
5. Blackwell CC, Weir DM, James VS, Cartwright KAV, Stuart JM, Jones DM. The Stonehouse study: secretor status and carriage of *Neisseria meningitidis* and *Neisseria lactamica*. Epidemiol Infect 1989; **102**: 1–10.
6. Blackwell CC, Weir DM. Meningococcal disease: high virulence and low transmission? Lancet 1990; ii: 53.
7. Mourant AE, Kopéc AC, Domaniewska-Sobczak K. The distribution of the human blood groups and other polymorphisms. London: Oxford University Press, 1976: 548–76.
8. Haneberg B, Tonjum T, Rodahl K, Gedde-Dahl T. Factors preceding the onset of meningococcal disease with special emphasis on passive smoking, stressful events, physical illness and general symptoms of ill health. Nat Inst Pub Health Annal 1983; **6**: 169–76.
9. Stuart JM, Cartwright KAV, Dawson AJ, Rickard J, Noah ND. Risk factors for meningococcal disease: a case control study in Southwest England. Comm Med 1988; **10**: 139–46.
10. Stuart JM, Cartwright KAV, Robinson PM, Noah ND. Effect of smoking on meningococcal carriage. Lancet 1989; ii: 723–5.
11. Krakinski K, Nilson JD, Butler S, Laby JP, Kusmiesz H. Possible association of mycoplasma in viral respiratory infections with bacterial meningitis. Am J Epidemiol 1987; **125**: 499–508.
12. Musher DM, Fainstein V. Adherence of *Staphylococcus aureus* to pharyngeal cells from normal subjects, smokers, staphylococcal carriers and patients with viral infections. In: Jeljaszewicz J, editor. Staphylococci and staphylococcal infections. New York: Gustav Fischer Verlag, Zbl Bakt Suppl 10, 1981: 1011–16.
13. Young H. Cultural diagnosis of gonorrhoea with modified New York City Medium (MNYC). Br J Vener Dis 1978; **54**: 36–40.
14. Pether JVS, Lightfoot NF, Scott RJD, Morgon J, Steele-Perkins AP, Sheard SC. Carriage of *Neisseria meningitidis*: investigations in a military establishment. Epidemiol Infect 1988; **101**: 21–4.
15. Cartwright KAV, Stuart JM, Jones DM, Noah MD. The Stonehouse survey: nasopharyngeal carriage of meningococci and *Neisseria lactamica*. Epidemiol Infect 1987; **99**: 591–601.
16. Mentis A, Blackwell CC, Weir DM, Spiliadis C, Dailianas A, Skandalis N. ABO blood group, secretor status and detection of *Helicobacter pylori* among patients with gastric or duodenal ulcers. Epidemiol Infect 1990; **106**: 221–9.
17. Hirszfeld L, Hirszfeld H. Serological differences between the blood of different races. The result of researches on the Macedonian front. Lancet 1919; ii: 675–9.
18. Crowdy JP. Smoking in the army. J Roy Army Med Corps 1981; **127**: 1–15.
19. Wald N, Kiryluk S, Darby S, Doll R, Pike M, Peto R. UK smoking statistics. Oxford: Oxford University Press, 1988.
20. Plotkowski M-C, Puchelle E, Beck G, Jacquot J, Hannoun C. Adherence of type I

Streptococcus pneumoniae to tracheal epithelium of mice infected with influenza A/PRB virus. Am Rev Resp Dis 1984; **134**: 1040–4.

21. Sanford B, Davison V, Ramsay M. *Staphylococcus aureus* adherence to influenza A virus infected and control cell cultures: evidence for multiple adhesions. Proc Soc Exp Biol Med 1986; **181**: 104–11.
22. Cartwright KAV, Jones DM, Smith AJ, Stuart JM, Kaczmarski EB, Palmer SR. Influenza A and meningococcal disease. Lancet 1991; **338**: 554–7.

Epidemiol. Infect. (1992), **108**, 449–455
Printed in Great Britain

Antibiotic sensitivities of *Neisseria meningitidis* isolates from patients and carriers in Greece

G. TZANAKAKI[1], C. C. BLACKWELL[2]*, J. KREMASTINOU[3], C. KALLERGI[4], G. KOUPPARI[4] AND D. M. WEIR[2]

[1] *National Meningitis Reference Laboratory, Hellenic Institute Pasteur, Athens, Greece*

[2] *Department of Medical Microbiology, The Medical School, University of Edinburgh, Edinburgh EH8 9AG*

[3] *Athens School of Public Health, Athens, Greece*

[4] *Aglaia Kyriakou Paediatric Hospital, Athens, Greece*

(*Accepted 10 November 1991*)

SUMMARY

Usage of antibiotics in southern Europe is less well regulated than in northern countries. The proportion (48%) of meningococci in Spain insensitive to penicillin (MIC $\geqslant$ 0·1 mg/l) prompted this investigation of antibiotic sensitivities of isolates from Greek patients with meningitis (31) and carriers (47 school-children and 472 recruits). The agar dilution method was used to determine MIC to penicillin G (PN), sulphamethoxazole (SU), rifampicin (RF), cefaclor (CF) and ciprofloxacin (CP).

The proportion of isolates insensitive to PN was 48% for isolates from patients, 19% from school-children and 36·6% from recruits. Resistance to SU (MIC $\geqslant$ 16 mg/l) was found in 16% of those from patients, 10·6% from children and 40% from recruits. None of the isolates from patients was resistant to RF ($\geqslant$ 1 mg/l) but 6% of those from carriers were. Resistance to CF ($\geqslant$ 4 mg/l) was found in 9·2% of patient isolates, 6·4% from children and 23·7% from recruits. All isolates except one were sensitive to CP (MIC range $<$ 0·0015–0·125 mg/l).

Resistances to PN, SU and RF were analysed by serogroup, serotype and subtype of the bacteria. The proportion of resistant isolates showed some variation between different areas of Greece, but it was not statistically significant.

INTRODUCTION

As part of the surveys of isolates of meningococci from patients and carriers in Greece, the antibiotic sensitivities of these bacteria to some common antibiotics were determined. There are reports of *Neisseria meningitidis* with reduced sensitivity to penicillin isolated from patients in Spain [1, 2], South Africa [3], and the United Kingdom [4, 5]. A small proportion of these penicillin-insensitive isolates has also been found among those obtained from carriers in the United Kingdom [4]. In view of the increasing prevalence of penicillin-insensitive isolates

* Author for correspondence.

in Spain and the similar antibiotic policies of both Spain and Greece, it was predicted that there might be significant numbers of Greek isolates with decreased sensitivity to penicillin and other antibiotics.

The objectives of the study were: (1) to compare levels of sensitivities for isolates obtained from patients with those obtained from carriers; (2) to determine if the proportion of penicillin-insensitive isolates is comparable to that reported for Spain; (3) to assess the distribution of resistant isolates in the different regions of Greece; (4) to determine if antibiotic resistances are associated with particular serogroups, serotypes or subtypes.

ISOLATES

Meningitis cases

Thirty-one isolates from children aged 1 month to 11 years were provided by the microbiology laboratory of the 'Aglia Kyriakou' Paediatric Hospital. All cases were sporadic and occurred during 1989–91.

Carriers

Four hundred and seventy-two isolates were obtained during 1990–1 from healthy recruits aged 16–31 years [6] and a further 47 from children aged 6–18 years who did not have meningococcal disease.

METHODS

Antibiotic sensitivities to penicillin (PN), erythromycin (ER), tetracycline (TC), sulphamethoxazole (SU), rifampicin (RF), cefaclor (CF) and ciprofloxacin (CP) were determined by the agar dilution method. A range of twofold dilutions from 256 to 0·015 mg/l of each antibiotic in Mueller Hinton medium was examined. The breakpoints used for penicillin ($\geq$ 0·125 mg/l), tetracycline ($\geq$ 1 mg/l), erythromycin ($\geq$ 0·5 mg/l), rifampicin ($\geq$ mg/l), ciprofloxacin ($\geq$ 1 mg/l) and the cephalosporin cefaclor ($\geq$ 4 mg/l) were based on those recommended by the working party of the British Society for Antimicrobial Chemotherapy for *Branhamella catarrhalis* and *Haemophilus influenzae* [7]. The break point of $\geq$ 10 mg/l for sulphamethoxazole (SU) was that used by the Neisseria Reference Laboratory (Scotland) [Dr R. J. Fallon, personal communication]. Beta-lactamase production was assessed during the rapid carbohydrate fermentation test for identification of each isolate [8].

Serogroup was determined by slide agglutination with commercially available antisera (Wellcome Diagnostics); serotype and subtype were determined by whole cell enzyme-linked immunosorbent assays with monoclonal antibodies from Dr J. T. Poolman (RIVM Bilthoven, The Netherlands) [9].

RESULTS

Table 1 compares the proportion of resistant isolates among the three groups examined. Among 31 isolates obtained from children with meningococcal disease, none was resistant to rifampicin or ciprofloxacin. Nearly half the isolates were penicillin-insensitive (48·3%) and all grew on 1 or 2 mg/l tetracycline.

Among the 47 isolates from school-children the pattern of resistance to

Table 1. *Proportion of antibiotic-resistant isolates among patients and carriers*

	Percent resistant		
		Carriers	
	Patients ($n = 31$)	Children ($n = 47$)	Recruits ($n = 472$)
Penicillin G	48·3	19·1	36·7
Sulphamethoxazole	16·1	10·6	35·6
Rifampicin	—	6·4	7
Tetracycline	100	6·4	91
Erythromycin	25·8	61·7	82·2
Cefaclor	9·7	6·4	23·7
Ciprofloxacin	—	—	0·2

ciprofloxacin, cefaclor and sulphamethoxazole was similar to that of isolates from patients. The proportion resistant to erythromycin (61·7%) was over twice that among isolates from patients (25·8%) ($\chi^2 = 8{\cdot}27$, df = 1, $P = < 0{\cdot}005$); and there were some rifampicin resistant strains (6·4%). Compared with the isolates from patients, the proportion resistant to tetracycline was much less (6·4%) ($\chi^2 = 62{\cdot}83$, df = 1, $P = < 0{\cdot}0005$) as was the proportion with decreased sensitivity to penicillin (19·1%) ($\chi^2 = 7{\cdot}61$, df = 1, $P = < 0{\cdot}01$).

The proportions of 472 isolates from recruits resistant to tetracycline or penicillin were similar to those of isolates from patients. The proportions of isolates from recruits resistant to erythromycin or to rifampicin were similar to that for isolates from children who were carriers. Compared with the other two groups, there was a higher proportion of isolates from recruits resistant to sulphamethoxazole and cefaclor. There were 112 isolates (23·7%) with MIC $\geqslant$ 4 mg/l cefaclor and 32 isolates (6·8%) with MIC $\geqslant$ 8 mg/l. Of the latter 32 isolates, 27 (84%) had decreased sensitivity to penicillin. There was one isolate with MIC $\geqslant$ 1 mg/l ciprofloxacin. None of the 550 isolates from patients and carriers produced beta-lactamase.

Geographical distribution of resistant isolates

In the two major population centres, Eastern Macedonia and Athens, the proportions of penicillin-insensitive isolates were 33 and 40% respectively. The highest proportions of such isolates were found in Thrace (50%), Crete (52%) and the Aegean and Ionian Islands (50%). The lowest proportion was obtained from recruits who lived abroad but who had returned to Greece for their compulsory military service (9%). Analysis of the serogroups, serotypes, subtypes and sensitivities to other antibiotics indicate that the isolates within a particular geographical area with decreased sensitivity to penicillin are not related (Table 2).

Analysis by antigenic phenotypes

Meningitis strains

Among the isolates from patients with meningitis, 50% of group B, 37·5% of group C and 60% of the non-groupables were penicillin-insensitive. Sulphonamide resistance was found in 17% of the group B isolates and 25% of group C but in

Table 2. *Proportion of antibiotic-resistant isolates obtained from recruits from different regions of Greece*

Region	Number tested	Percent resistant			
		PN	SU	RF	CP
Thrace	14	50	79	7	—
W. Macedonia	38	26	42	5	—
E. Macedonia	96	33	35	7	—
Epirus	23	48	30	4	—
Thessaly	46	34	48	6	—
Central Greece	32	32	44	9	—
Athens	127	40	39	7	0·8
Peloponnese	40	35	30	10	—
Crete	31	52	39	3	—
Aegean Islands	10	50	70	10	—
Ionian Islands	2	50	—	50	—
Foreign	11	9	22	—	—

Table 3. *Antibiotic resistances of serogroups*

Serogroup	No.	Percent resistant		
		PN	SU	RF
A	17	41	41	12
B	114	35	37	5
C	19	42	42	10·5
W135	2	0	50	0
Y	8	62·5	25	0
Z	4	25	0	0
NG	300	36	40	7

none of the non-groupable isolates. Of the 9 2b serotype isolates, 7 (78%) were penicillin-insensitive as were 8/18 (44%) of the non-typables. The 4 isolates expressing other serotypes (2a, 14 and 15) were sensitive to penicillin and to sulphamethoxazole. Resistance to sulphamethoxazole was found for only 2/9 serotype 2b isolates and 3/18 non-typable isolates. Of the 9 isolates expressing the P1.10 subtype, 7 (78%) were penicillin-insensitive though only 1 was resistant to sulphamethoxazole. The second most common subtype was P1.9. Only 1 of the 5 P1.9 isolates was penicillin-insensitive, but 2 were sulphonamide resistant.

Carrier strains

There were sufficient numbers of isolates from the recruits for analysis of antibiotic resistances by serogroup, serotype and subtype (Tables 3–5). Among the serogroups, the highest proportion of penicillin-insensitive isolates was found for group Y (62·5%) while rifampicin resistance was greatest in groups A (12%) and C (10·5%) (Table 3).

Penicillin-insensitivity was highest among serotypes 2a and 15 and lowest in type 4, which, however, had the highest proportion of strains resistant to sulphamethoxazole. Rifampicin resistance was observed most frequently among serotypes 4 and 15 (Table 4).

Table 4. *Antibiotic resistances among serotypes*

Serotype	No.	Percent resistant PN	SU	RF
1	15	27	20	7
2a	7	57	43	0
2b	22	27	32	4·5
4	9	11	56	11
14	66	42	28	9
15	16	56	19	12·5

Table 5. *Antibiotic resistances among subtypes*

Serogroup	No.	Percent resistant PN	SU	RF
1	41	39	34	2·5
2	72	43	22	15·5
4	7	43	29	0
6	28	32	68	0
7	30	43	63	13
9	45	27	38	2·4
10	29	45	28	14
12	8	25	37·5	0
14	8	62·5	25	0
15	16	37·5	19	0
16	13	38	70	23

No particular subtype antigen was associated with an unusual degree of antibiotic resistance (Table 5).

DISCUSSION

Although sulphonamide resistance has been associated with strains causing outbreaks of meningococcal disease in northern Europe [10, 11], only 16% of the isolates from Greek patients had MIC > 10 mg/l to sulphamethoxazole; however, nearly half had reduced sensitivity to penicillin and all grew in the presence of 1 or 2 mgm/l tetracycline.

In contrast, while the proportions of penicillin-insensitive and of tetracycline-resistant isolates from carrier children were both significantly lower, the proportion of erythromycin isolates was significantly increased.

Among isolates from the recruits, the proportion of penicillin-insensitive strains was significantly greater than that from children who were carriers ($\chi^2 = 5{\cdot}126$, df = 1, $P < 0{\cdot}025$) but not compared with those from children with disease. The proportion of sulphonamide-resistant isolates among those from recruits (35·6%) was over twice that of the other two groups, carriers (10·6%) ($\chi^2 = 10{\cdot}882$, df = 1, $P < 0{\cdot}005$) or patients (16%) ($\chi^2 = 4{\cdot}06$, df = 1, $P < 0{\cdot}05$) (Table 1).

Although penicillinase-producing isolates of *Neisseria gonorrhoeae* are not uncommon in Greece (18%) [12], none of the 550 meningococcal isolates tested produced beta-lactamase.

isolated. Although the sulphonamide and rifampicin resistances were highest among subtype P1.16, the two resistances were not found together in the P1.16 strains. The higher rate of rifampicin resistance among isolates expressing P1.10, the subtype found most frequently among meningococci isolated from patients, and among isolates expressing P1.2, the most common subtype among carriers, needs to be further investigated.

REFERENCES

1. Saez-Nieto JA, Fontanals D, Garcia de Jalon J, et al. Isolation of *Neisseria meningitidis* strains with increase of penicillin minimal inhibitory concentration. Epidemiol Infect 1987; **99**: 463–9.
2. Saez-Nieto JA, Vazquez JA, Macros C. Meningococci moderately resistant to penicillin. Lancet 1990; ii: 54.
3. Botha P. Penicillin-resistant *Neisseria meningitidis* in southern Africa. Lancet 1988; i: 54.
4. Sutcliffe EM, Jones DM, El-Sheikh S, et al. Penicillin-insensitive meningococci in the UK. Lancet 1988; i: 657–8.
5. Jones DM, Sutcliffe EM. Meningococci with reduced susceptibility to penicillin. Lancet 1990; i: 863–4.
6. Blackwell CC, Tzanakaki G, Kremastinou J, et al. Factors affecting carriage of *Neisseria meningitidis* among Greek military recruits. Epidemiol Infect 1992; **108**: 441–8.
7. AAC working party. Phillips I, Andrews J, Bint AJ, et al. Breakpoints in *in vitro* antibiotic sensitivity testing. J Antimicrob Chemother 1988; **21**: 701–10.
8. Young HY. Identification and penicillinase testing of *Neisseria gonorrhoeae* from primary isolation cultures on MNYC medium. J Clin Microbiol 1978; **7**: 247–50.
9. Abdillahi H, Poolman JT. Whole-cell ELISA for typing *Neisseria meningitidis* with monoclonal antibodies. J Med Microbiol 1987; **48**: 367–71.
10. Cartwright KAV, Stuart JM, Jones DM, Noah ND. The Stonehouse survey: nasopharyngeal carriage of meningococci and *Neisseria lactamica*. Epidemiol Infect 1987; **99**: 591–601.
11. Poolman JT, Jonsdóttir K, Jones DM, Lind I, Frøholm O, Zanen HC. Meningococcal serotypes and serogroup B disease in Northwest Europe. Lancet 1986; ii: 555–8.
12. Tzanakaki G, Mavromati L, Tzelepi E, et al. Serological classification in relation to auxotypes, plasmid contents, and susceptibility to antimicrobials of PPNG and non-PPNG strains isolated in Greece. Genitour Med 1989; **65**: 171–6.
13. Gaunt PM, Lambert BE. Single dose ciprofloxacin for eradication of pharyngeal carriage of *Neisseria meningitidis*. J Antimicrob Chemother 1988; **21**: 489–96.
14. Knapp JS, Johnson SR, Zenilman JH, et al. High-level tetracycline resistance resulting from TetM in strains of *Neisseria* spp., *Kingella denitrificans* and *Eikenella corrodens*. Antimicrob Agents Chemother 1988; **32**: 765–7.

J Clin Pathol 1992;45 (Supplement) 20-24

Susceptibility to infection in relation to SIDS

C C Blackwell, A T Saadi, M W Raza, J Stewart, D M Weir

Summary

Because there is little evidence that invasive bacterial diseases contribute to cot deaths, most studies on infectious causes of SIDS have focused on viruses or toxin producing bacteria.

Although epidemiological studies found marginally significant associations between influenza virus and SIDS, respiratory syncytial virus (RSV) was isolated from 90% of older infants with SIDS. There are conflicting reports that some toxigenic bacteria *(Clostridium botulinum, Clostridium difficile,* enterotoxigenic *Escherichia coli* and *Staphylococcus aureus)* might be implicated in cot deaths. *S aureus* are common micro-organisms and their toxins are very powerful. As the pyrogenic toxic shock syndrome toxin of *S aureus* can kill a previously healthy adult, it might easily kill a small infant.

Based on our studies on susceptibility of infants to other infections, we suggest the following might be factors leading to colonisation of infants by toxin producing *S aureus*:

- **The Lewis^a blood group antigen appears to act as a receptor for some micro-organisms. Epithelial cells expressing high concentrations of Lewis^a bound appreciably more toxin producing *S aureus* than cells expressing low concentrations of the antigen.**
- **Lewis^a is expressed in secretions of nearly 90% of infants aged 3 months, the peak age for SIDS.**
- **RSV infects about 50% of infants by the first year of life and it is often isolated from infants with SIDS. Studies in our laboratory indicate that RSV infected HEp-2 cells bind more toxin producing *S aureus* than uninfected controls.**

For many years it has been suggested that micro-organisms might be involved in the aetiology of SIDS; several of the factors identified in epidemiological studies of SIDS are associated with increased susceptibility of infants to infectious diseases. SIDS is associated with the vulnerable period when maternal antibodies have decreased, and the infant's immune system is immature. Deaths from SIDS occur more frequently during the winter months when respiratory viral infections are most prevalent. Both occur frequently in families in which socio-economic conditions are poorer. One of the factors identified in the New Zealand studies on cot death was maternal smoking habit.[1] Smoking is, however, related to socio-economic group. In the United Kingdom the proportion of women who smoke increases as social class decreases from professional to partly skilled or unskilled categories.[2] Smoking and passive exposure to cigarette smoke have been associated with susceptibility to respiratory infections.[3] Smokers are also more likely to be asymptomatic carriers of potential pathogens such as meningococci.[4,5] Breast feeding protects infants in this age range from gastrointestinal and respiratory illnesses, and SIDS is claimed to be more frequently associated with bottle feeding.[6]

Viruses

Much of the work on the role of infectious agents in SIDS has concentrated on viruses. Various respiratory viruses have been implicated; chapters 6 and 7 provide critical reviews of the evidence for viral infection as a cause of SIDS. Although viruses have been isolated from infants with SIDS, it has been difficult to establish a direct link for viral infection with these deaths.

Bacteria

Generally, bacteria cause disease in two ways, by invading the host or by producing a toxin. There has been no substantial evidence for invasive bacterial infections in SIDS.[7,8] Postmortem studies have isolated several toxin producing bacteria from these infants (table 1). Infant botulism has been reported to be responsible for 4% of cot deaths in the United States and up to 16% of those in Sweden.[9-12] This pattern has not been found in the United Kingdom.[13]

Toxins A and B of *C difficile* produce death in monkeys that is pathologically consistent with SIDS,[14] and these bacteria have been isolated from some infants at necropsy.[15,16] Enterotoxigenic strains of *E coli* have also been implicated in some studies[15]; the evidence for the involvement of intestinal toxigenic bacteria has, however, been inconsistent.

Table 1 Toxin producing bacteria implicated in SIDS

Species	*Toxin*	*Reference*
C botulinum	A, B, C, F, G	6 11-14
C difficile	A, B	16-18
E coli	Enterotoxin	17
S aureus	A, B, C, D, TSST-1*	19 22

*Toxic shock syndrome.

Table 2 Blood group antigens on cells and in body fluids of secretors and non-secretors

	Secretors		Non-secretors	
BG antigen	Cells	Secretions	Cells	Secretions
H (A/B)	+	+	+	-
Lewis[a]	-(+)*	-(+)*	+	+
Lewis[b]	+	+	-	-

*Present in variable quantities.

The pyrogenic toxins of *Staphylococcus aureus* such as that associated with toxic shock syndrome can kill previously healthy adults, so they might easily kill a small infant.[17] One of the characteristics of disease caused by these toxigenic bacteria is high fever (>38·5°C). Among 24 infants who died suddenly and whose rectal temperatures were measured immediately before refrigeration, 10 had temperatures above 38°C and five above 40°C.[18]

The pyrogenic toxins produced by the Gram positive bacteria *S aureus* and group A *Streptococcus pyogenes* have similar properties. They are potent inducers of fever, perhaps by direct action on the hypothalamus or through their induction of interleukin 1 (IL-1) and tumour necrosis factor (TNF) from mononuclear phagocytes. These toxins are mitogenic for lymphocytes. They can non-specifically suppress immunoglobulin production and enhance delayed type hypersensitivity; these are probably not important functions in SIDS, however, as the infants have little specific immunity at this time. The toxins can alter liver clearance function and enhance endotoxic shock. An important fact to note is that they are produced at 37–40°C, and more toxin is produced at the higher temperatures.[19]

The study by Telford and colleagues published in 1989[20] led us to consider how toxin producing strains of *S aureus* might cause some cot deaths. Although the proportion of infants with SIDS from whom *S aureus* was isolated was greater than the controls, the difference was not significant. The proportion of streptococci isolated from infants with SIDS was significantly higher; none of these, however, was a group A *S pyogenes* which produces pyrogenic toxins similar to those of staphylococci. A variety of toxin producing staphyloccoci, including that associated with toxic shock syndrome, have been isolated from infants with SIDS.[20] We chose these bacteria as the model for our investigations.

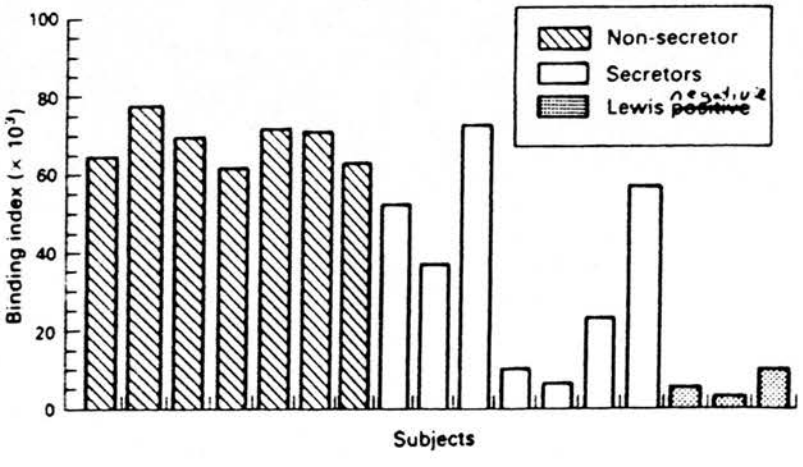


Figure 1 Binding of monoclonal anti-Lewis[a] antibody to buccal epithelial cells from non-secretors, secretors and Lewis-negative donors.

Secretor status and susceptibility to infectious agents

Our research team has been examining a genetic factor associated with the susceptibility of individuals to several infectious diseases. This is non-secretion, the inability of an individual to secrete the glycoprotein form of his ABO blood group antigens. The first association between non-secretion and infectious diseases was noted in the 1950s and 1960s among patients with rheumatic fever. Non-secretors were over-represented among patients with rheumatic fever and also among asymptomatic carriers of group A *S pyogenes*.[21]

Traditionally, secretor status is determined by detection of ABO blood group antigens in saliva by a haemagglutination inhibition test, but it can also be determined by the Lewis blood group antigen. Non-secretors can produce only Lewis[a]; while secretors can have varying quantities of Lewis[a], they predominantly express Lewis[b].[22] We have developed enzyme linked immunosorbent assays (ELISA) for detection of H antigen[23] and Lewis antigens[24] in body fluids such as saliva or nasal secretions so that, even if we do not have red blood cells, we can still determine the Lewis blood group of a subject. The distribution of ABO and Lewis blood group antigens on cells and in body fluids of secretors and non-secretors is outlined in table 2.

One hypothesis we suggested to explain the increased susceptibility of non-secretors to some infectious agents was that the Lewis[a] antigen, present in greater quantities on epithelial cells of non-secretors, might act as a receptor for some strains of bacteria.[25] The idea is not novel; a variety of blood group antigens have been shown to act as receptors for micro-organisms. For example, the Duffy blood group acts as a receptor for *Plasmodium vivax* in populations expressing this antigen.[26]

By flow cytometry we measured the amount of monoclonal anti-Lewis[a] antibody bound to epithelial cells from non-secretors, secretors and subjects who lack the Lewis gene. Cells from non-secretors consistently bound high concentrations of anti-Lewis[a]; those from secretors bound highly variable amounts; while those from Lewis-negative subjects consistently bound very low amounts of the antibody (fig 1).

The peak of SIDS at 2–3 months of age suggests that cot deaths might be associated with a critical phase of development during which either all or a subgroup of babies are at risk.[27] There are differences in maturation or efficiency of the products of the secretor gene and the Lewis gene during this period in the infants' development.

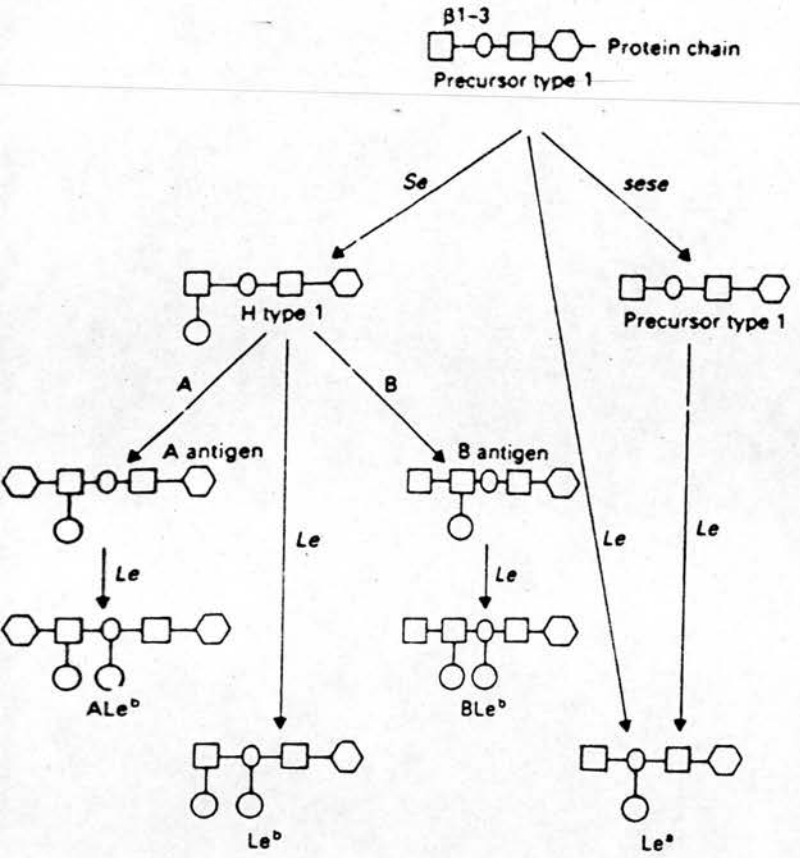


Figure 2 Derivation of ABH and Lewis antigens from type 1 precursor chains.

The enzymes coded for by the secretor and Lewis genes act on type 1 precursor chains attached for the most part to glycoproteins in body fluids. The enzyme coded by the secretor gene adds fucose to the terminal sugar of the type 1 precursor and that coded for by the Lewis gene adds fucose to the subterminal sugar of the type 1 precursor. If the secretor enzyme adds the fucose first, the structure can act as a substrate for the Lewis enzyme to form Lewis^b antigen. If the Lewis enzyme adds fucose to the subterminal sugar first to form Lewis^a, the secretor gene cannot use the Lewis^a as a substrate to form Lewis^b (fig 2). In infants the enzyme coded by the secretor gene does not mature as rapidly as the one coded by the Lewis gene. The result is that infants, even though they are secretors, express considerable amounts of Lewis^a on their cells.[24]

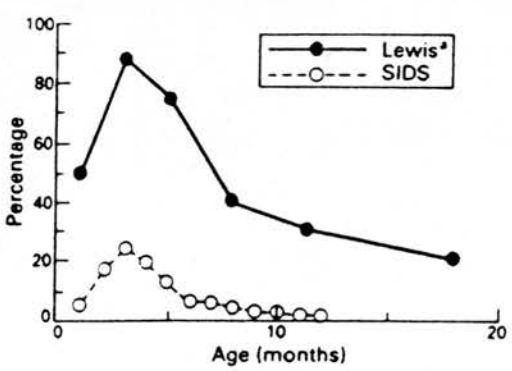


Figure 3 Analysis by age of expression of Lewis^a antigen and prevalence of SIDS.

When we looked at the figures published for percentage of infants expressing Lewis^a, it was obvious that the curve mirrored that for SIDS (fig 3). When we examined secretions from 89 infants with SIDS by the ELISA method, we found Lewis^a antigen in 63 (71%).

In a series of experiments we tested the hypothesis that some strains of toxin producing *S aureus*. bind in greater numbers to cells expressing large amounts of Lewis^a. Because we could not use cells from infants, we examined epithelial cells from adult secretors and non-secretors matched as closely as possible for ABO blood group, sex, and age for their abilities to bind strains of *S aureus* obtained from different sources (table 3). Attachment of fluorescein labelled bacteria to buccal epithelial cells obtained from matched pairs of secretor and non-secretor donors was assessed by flow cytometry[10] and analysed by the computer program Immunoanalysis (Coulter). The binding index of each sample (percentage of fluorescent cells multiplied by the mean fluorescence) was calculated.

In figs 4 and 5 the data are represented as a difference in the percentage of the binding between secretor and non-secretor cells, each point representing the results for one pair of donors. If there was no difference the point is on the line (0%). The points above the line represent those pairs in which the non-secretor cells bound more bacteria than cells from the secretor: the points below the line represent those pairs in which the secretor cells bound more than those from the non-secretor.

Five of the eight toxin-producing strains and the non-toxigenic strain showed no difference in binding to cells of secretors compared with non-secretors. Figure 4 represents the type of pattern observed with these strains. For three of the toxigenic strains, including one producing TSST-1, there was significantly more binding to the cells on non-secretors (fig 5). This indicates that some but not all strains bind in greater numbers to cells of non-secretors.

If some toxin-producing strains use Lewis^a as one of the host cell receptors, the results suggest that at the age of 2 to 3 months infants might be more susceptible to colonisation by these bacteria.

Interactions between viruses and bacteria

There is clinical, epidemiological, and experimental evidence that virus infections predispose people to some bacterial diseases and enhance colonisation by potentially pathogenic

Table 3 Staphylococcus aureus isolates examined

Strain number	*Toxin produced*	*Source*
8532	—	
19655*	A, TSST-1	Vaginal swab
10655*	C	Leg abscess
10657*	A & B	
10656	D	Turkey salad
10654	B	Faeces
10652	A	Ham
40654	A	Nose
41206	B	Postmortem lung tissue

*Increased binding to non-secretor cells.

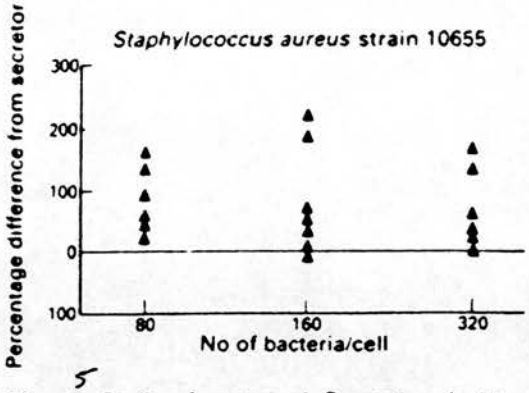


Figure 5 Binding of toxigenic S aureus to epithelial cells of secretors and non-secretors.

bacteria, such as influenza followed by invasive pneumococcal disease. Some of the interactions that might lead to secondary bacterial disease include: immunosuppression; decreased phagocytosis; loss of mucociliary function; local oedema formation and tissue injury; and increased binding to virus infected cells.

Toxic shock syndrome caused by toxin producing staphylococci has been identified as a complication of influenza or influenza-like illness.[29] In our studies we tested the hypothesis that cells infected with RSV bind more bacteria than uninfected cells. We chose RSV as a model system for these studies because it is an important, ubiquitous respiratory pathogen causing infection in young infants during the winter months when SIDS is most common.[30] It has been isolated often from infants with SIDS.[31] By the age of 12 months half of all infants have been infected with the virus. It can be grown in an epithelial cell line of human origin. Finally, our studies on two bacterial species that cause invasive disease among children in the age range affected by SIDS, *Neisseria meningitidis* and type b *Haemophilus influenzae*, found that cells infected with RSV bound significantly more of these bacteria than uninfected cells (Raza MW, Ogilvie MM, Blackwell CC, *et al.* Abstract presented at 120th meeting of Society for General Microbiology).

A flow cytometry method was adapted to examine attachment of bacteria to tissue culture cells derived from human epithelial cells (HEp2) cells which can be infected with RSV. The results from our preliminary experiments with staphylococci closely paralleled those found with the other two species of bacteria. Compared with the uninfected cells the infected cells bound more staphylococci with both the toxin producing strain (10655) and the non-toxigenic strain tested (8325) (fig 6).

If toxin producing staphylococci are responsible for some of these infant deaths, our studies suggest two factors that might contribute to colonisation by these bacteria: expression of abnormally high concentrations of Lewis^a in infants under the age of 12 months; and enhanced binding of these bacteria to infected cells. The following scheme attempts to incorporate the factors identified in epidemiological studies with our experimental work.

First, maternal smoking, which has been identified as a risk factor for SIDS, could enhance exposure of the infant to staphylococci in two ways. Epithelial cells from smokers bind significantly more staphylococci than cells from non-smokers.[32] Smoking also enhances susceptibility to respiratory viral infections; epithelial cells from patients with natural or experimental viral infections bound more staphylococci compared with cells from people who were not infected with a virus.[32]

Factors that might enhance colonisation of infants by toxigenic staphylococci include passive exposure to cigarette smoke which decreases mucocillary clearance. Infants in the age range affected by SIDS have little or no mucosal or systemic immunity to staphylococci or their toxins. The effect of breast feeding on colonisation by staphylococci is not known, but breast fed infants are less susceptible to toxigenic strains of *C botulinum*.[6] Infants in the 2–3 month age group express large amounts of Lewis^a, which we postulate, some isolates of toxigenic staphylococci might use as a receptor on epithelial cells. Respiratory viral infections have been shown to enhance binding of staphylococci to epithelial cells from 8% among uninfected people to 43% among those with infection[33]; our laboratory finding indicate that the common paediatric virus, RSV, can also enhance binding to epithelial cells in vitro.

In most infants colonisation by staphylococci does not result in SIDS; additional

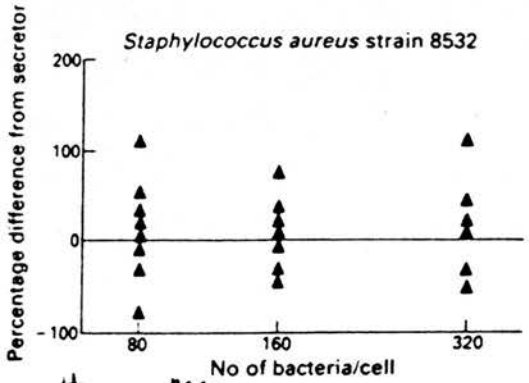


Figure 4 Binding of toxigenic S aureus to epithelial cells of secretors and non-secretors.

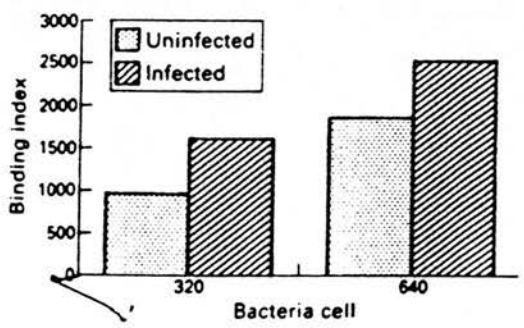


Figure 6 Binding of toxin-producing S aureus (10655) to uninfected HEp-2 cells and HEp-2 cells infected with RSV.

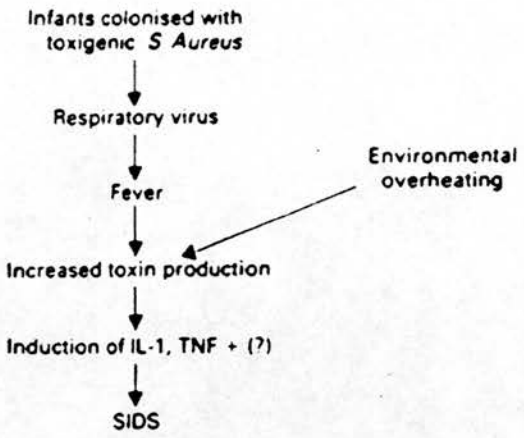


Figure 7 Proposed series of events by which bacteria producing pyrogenic toxins might cause some cot deaths.

factors are probably required to precipitate cot death. In some infants the series of events outlined in fig 7 might occur. The temperature of the infant colonised by the toxigenic bacteria is increased, whether by virus infection or by environmental overheating. The higher temperature enhances the amount of toxin produced by the bacteria,[19] and the toxin diffuses into the bloodstream to increase the temperature of the infant further. This synergistic effect between increased temperature and toxin production might account for the unusually high temperatures recorded for some of these infants at necropsy.[18] The actual cause of death might be shock due to the fever or other as yet unrecognised effects of the toxin on the respiratory or cardiac systems.

Our studies suggest three areas for further investigation. The first is already underway: to identify the receptors on the cells of non-secretors that contribute to the enhanced binding of staphylococci and the components on RSV infected cells that enhance binding of bacteria. From this we will try to identify the adhesions on the bacteria and to determine whether they can elicit an immuned response in infants in this vulnerable age group. The second set of studies requires development of assays for detection of pyrogenic toxins to determine whether these are found in some infants with SIDS at necrospy. Last is epidemiological studies of the effects of parental smoking and viral infections on the carriage of staphylococci among infants during the first year of life, which we are trying to organise in conjunction with our studies on meningitis.

Toxigenic bacteria might have a role in some cot deaths. Colonisation by toxigenic strains is probably not uncommon. In most infants this colonisation does no harm and induces protective antibodies to the bacteria. We suggest that in most instances in which these pyrogenic toxins might be involved, there must be other factors such as viral infection or overheating that create conditions in which these bacteria produce enough toxin to have a lethal effect. The studies planned will allow us to obtain evidence to support or refute the hypothesis.

1 Mitchell EA. Cot death: should the prone sleeping position be discouraged? *J Paediatr Child Health* 1991; 27:319–21.
2 Wald N, Karyluk S, Darby S, *et al. UK smoking statistics 1988.* Oxford: Oxford University Press, 1989
3 Bonham GS, Wilson RW. Childrens health in families with cigarette smokers. *Am J Public Health* 1981;71:290–3.
4 Stuart JM, Cartwright KAV, Robinson PM, Noah ND. Effect of smoking on meningococcal carriage. *Lancet* 1989;ii:723–5.
5 Blackwell CC, Weir DM, James VS, *et al.* Secretor status, smoking and carriage of Neisseria meningitidis. *Epidemiol Infect* 1990;104:203–9.
6 Arnon SS. Breast feeding and toxigenic intestinal infections: missing links in crib death? *Rev Infect Dis* 1984;6 (suppl):S193–201.
7 Banks AL. An enquiry into sudden death in infancy. *Month Bull Ministry Hlth* 1958;17:182–91.
8 Valdes-Dapena MA. Sudden and unexpected death in infancy: a review of the world literature 1954–1966. *Pediatrics* 1967;39:123–38.
9 Arnon SS, Midura TF, Damus K, Wood RM, Chin J. Intestinal infection and toxin production by Clostridium botulinum as one cause of sudden infant death syndrome. *Lancet* 1978;i:1273–7.
10 Arnon SS, Damus K, Chin J. Infant botulism: epidemiology and relation to sudden infant death syndrome. *Epidemiol Rev* 1981;3:45–66.
11 Sonnabend OAR, Sonnabend WFF, Krech U, Molz G, Sigrist T. Continuous microbiological and pathological study of 70 sudden and unexpected infant deaths: toxigenic intestinal Clostridium botulinum infection in 9 cases of sudden infant death syndrome. *Lancet* 1985;i:237–41.
12 Anonymous. Infant botulism [Editorial]. *Lancet* 1986; ii:1256–7.
13 Turner HD, Brett EM, Gilbert RJ, Ghosh AC, Liebeschuetz HJ. Infant botulism in England. *Lancet* 1978;i:1277–8.
14 Arnon SS, Mills DC, Day PA, *et al.* Rapid death of infant rhesus monkeys injected with Clostridium difficile toxins A & B: physiologic and pathologic basis. *J Pediatr* 1984;104:34–40.
15 Gurwith MJ, Langston C, Citron DM. Toxin producing bacteria in infants. Lack of an association with sudden infant death syndrome. *Am J Dis Child* 1981; 135:1104–6.
16 Cooperstock MS, Steffen E, Yolken R, Onderdonk A. Clostridium difficile in normal infants and sudden infant death syndrome: an association with infant formula feeding. *Pediatrics* 1982;70:91–5.
17 Morris JA, Haran D, Smith A. Hypothesis: common bacterial toxins are a possible cause of the sudden infant death syndrome. *Med Hypotheses* 1987;22:211–22.
18 Sunderland R, Emery JL. Febrile convulsions and cot death. *Lancet* 1981;ii:176–8.
19 Bohach GA, Fast DJ, Nelson RD, Schlievert PM. Staphylococcal and streptococcal pyrogenic toxins involved in toxic shock syndrome and related illnesses. *Crit Rev Microbiol* 1990;17:251–72.
20 Telford DR, Morris JA, Hughes P, *et al.* The nasopharyngeal bacterial flora in the sudden infant death syndrome. *J Infect* 1989;18:125–30.
21 Haverkorn MJ, Goslings WRO. Streptococci, ABO blood groups and secretor status. *Am J Hum Genet* 1969; 21:360–75.
22 Issit PD. *Applied blood group serology.* 3rd edn Miami: Montgomery. 169–91.
23 Rahat A, Stewart J, Blackwell CC, Weir DM. Semi-quantitative determination of H type 1 and type 2 antigens on buccal epithelial cells and in saliva of secretors and non-secretors. *Vox Sang* 1990;59:101–5.
24 Raza MW, Blackwell CC, Molyneaux P, *et al.* Association between secretor status and respiratory viral illness. *Br Med J* 1991;303:815–18.
25 Blackwell CC. The role of ABO blood groups and secretor status in host defences. *FEMS Microbiol Immunol* 1989;47:341–50.
26 Miller LH, Mason SJ, Dvorak JA, McGinniss MH, Rothman IK. Erythrocyte receptors for (Plasmodium knowlesi) malaria: Duffy blood group determinants. *Science* 1975;189:561–3.
27 Milner AD. Recent theories on the cause of cot death. *Br Med J* 1987;295:1366–8.
28 Rahat A. Studies on the role of blood group antigens in susceptibility to meningococcal infection. PhD Thesis. University of Edinburgh, 1990.
29 McDonald KL, Osterholm MT, Hedberg CW, *et al.* Toxic shock syndrome. A newly recognised complication of influenza and influenze-like illness. *JAMA* 1987; 257:1053–8.
30 Noah ND. Cyclic patterns and predictability in infection. *Epidemiol Infect* 1989;102:175–90.
31 Williams AL, Uren EC, Bretherton L. Respiratory viruses and sudden infant deaths. *Br Med J* 1984;288:1491–3.
32 Musher DM, Fainstein V. Adherence of *Staphylococcus aureus* to pharyngeal cells from normal subjects, smokers, staphylococcal carriers and patients with viral infection. In: Staphylococci and staphylococcal infections. *Zbl Bakt* 1981;Suppl 10:1011–6.
33 Ramfrez-Ronda CH, Fuxench-Lopez A, Nevarez M. Increased bacterial colonisation during viral illnes. *Arch Intern Med* 1981;141:1599–603.

J. Med. Microbiol. — Vol. 38 (1993), 19–22

Serogroups, serotypes and subtypes of *Neisseria meningitidis* isolated from patients and carriers in Greece

G. TZANAKAKI, C. C. BLACKWELL*, J. KREMASTINOU†, D. M. WEIR*, A. MENTIS and R. J. FALLON‡

*National Meningitis Reference Laboratory, Hellenic Institute Pasteur, Athens, Greece, *Department of Medical Microbiology, University of Edinburgh Medical School, Teviot Place, Edinburgh EH8 9AG, †Athens School of Public Health and ‡Meningococcal Reference Laboratory (Scotland), Ruchill Hospital, Glasgow*

Summary. The increase in the number of cases of meningococcal disease reported to the Ministry of Health in Athens since 1989 prompted the present study to determine if isolates from patients or carriers expressed the same phenotypic characters as those in other parts of Europe. None of the isolates from patients (31) or carriers (547) expressed the antigenic combinations associated with outbreaks in northern Europe, i.e., B:15:P1.16 or B:4:P1.15. The majority of the Greek isolates did not react with any of the six monoclonal serotype reagents tested; however, most reacted with one or more of the 11 monoclonal subtype antibodies. The results suggest that additional serotype reagents are needed for epidemiological studies in southeastern Europe and that vaccines based on serotype antigens developed against outbreak strains in northern Europe would not be effective in Greece.

Introduction

The increase in the number of cases of meningococcal disease reported to the Ministry of Health in Athens prompted the present study.[1] As the last major epidemiological survey of *Neisseria meningitidis* in Greece was published in the 1970s before the availability of serotype and subtype reagents,[2] the study had three main objectives: firstly, to determine whether the serotype and subtype reagents used in northern Europe and America can discriminate between isolates of meningococci from south-eastern Europe; secondly, to find out if serogroups, serotypes and subtypes of isolates from patients with meningococcal disease or carriers in northern Europe[3–7] are prevalent among Greek isolates; and thirdly to see if particular phenotypes are associated with different regions of Greece.

Materials and methods

Thirty-one isolates from children aged 1 month–11 years with meningococcal disease were obtained from the microbiology laboratory of Aglaia Kyriakou Paediatric Hospital, Athens. The isolates were from sporadic cases that occurred during 1989–1991. Fifty-one isolates from school children aged 5–18 years who were carriers were obtained in surveys undertaken in Athens between Dec. 1990 and Jan. 1991.[8] A series of 496 isolates from military recruits aged 16–30 years were obtained during July 1990 and Jan. 1991[1] within 3–5 days of the arrival of the recruits at the camps; recruits from all regions of Greece were represented.

The isolates were grown on modified New York City medium[9] and characterised by Gram's stain, the oxidase test and by the rapid carbohydrate utilisation test (RCUT).[10] Serogroups were determined by slide agglutination with polyclonal antisera to serogroups A, B, C, W135, X, Y and Z (Wellcome Diagnostics). Serotype and subtype were determined by a whole-cell enzyme-linked immunoassay (ELISA) with monoclonal antibody[11] reagents supplied by Dr J. T. Poolman (RIVM, Bilthoven, The Netherlands).

Information obtained from each recruit regarding area of residence and socio-economic status was coded and entered into a Data Base 3 Plus program together with the results of the throat swab and the serogroup, serotype and subtype of the isolates.[1] Similar data bases were prepared for information obtained from questionnaires returned by parents of school children participating in the surveys.[8] Written informed consent was obtained from each of the recruits or from the parent or guardian of the children.

The results from the school children were compared with those obtained in a Scottish secondary school, where there was an outbreak of disease caused by a sulphonamide-resistant serogroup B serotype 4 subtype 15 (B:4:P1.15) strain,[7] and results from the recruits were compared with published results for 133

Received 4 March 1992; accepted 21 April 1992.
*Correspondence should be sent to Dr C. C. Blackwell.

isolates obtained from carriers among Norwegian military recruits.[6]

Results

Patients

The antigenic phenotypes of isolates from patients are shown in table I. The most prevalent serogroup was B (58%) followed by group C (26%) and non-groupable isolates (15%). The majority of isolates (58%) did not react with any of the serotype antibodies. The most common serotype was 2b (9 of 31, 29%), and the most common subtype was P1.10 (11 of 31, 35%). The most common serotype-subtype combination was 2b:P1.10 (7 of 31, 23%).

Carriers

Among school children there was no predominant antigenic phenotype. Of the 19 (37%) serogroupable isolates, 10 belonged to group B, two to each of serogroups A, C, W135 and Y and one to group Z. As with the isolates from patients, the majority (65%) did not react with any of the serotype antibodies; however, most (75%) reacted with one or more subtype reagents. Two isolates (4%) obtained from secondary school pupils expressed the 2b:P1.10 combination found among those from patients (tables II and III).

Of the 496 isolates from recruits, 36% were serogroupable: 24% were group B, 5% group C and 4% group A. Most isolates (69%) were not serotypable, but 64% reacted with one or more of the subtype antibodies. The most common serotype was 14 (16%), and the most common subtype was P1.2 (16%) (tables II and III). The most prevalent combination of serotype-subtype antigens was 14:P1.2 ($n = 24$) followed by 14:P1.7 ($n = 13$). None of the isolates from the recruits expressed the 2b:P1.10 combination predominant among children with meningococcal disease. None of the 2a or 2b isolates from carriers reacted with any of the subtype antibodies.

Geographic areas

The major regions of Greece are shown in the figure together with the proportion of recruits from whom meningococci were isolated. Meningococci were obtained most frequently from recruits from the north-east of Greece: Thrace (37%), West Macedonia (33%) and East Macedonia (30%). Because military service is compulsory, even for individuals who live abroad, there were 33 subjects who were not normally resident in Greece. Among these, 33% were carriers.

There were no unusual distributions of serogroups among the different regions. The most common serotype (14) was found in equal proportions (15%) among the isolates from the two major centres of

Table I. Antigenic phenotypes of isolates from children with meningococcal disease

Antigenic phenotype	Number of isolates
B:-:-	3
B:-:P1.1	1
B:-:P1.9	4
B:-:P1.10	2
B:-:P1.14	1
B:2b:P1.9	1
B:2b:P1.10	4
B:14:P1.15	1
B:15:P1.6	1
C:-:P1.6	2
C:-:P1.10	3
C:-:P1.14	1
C:-:P1.16	1
C:2b:P1.2	1
NG:2a:P1.6	1
NG:2b:P1.10	3
NG:14:P1.10	1

Table II. Comparison of serotypes of isolates from carriers in Greece with those from carriers in northern Europe

Serotype	Percentage of isolates from			
	recruits		school children	
	Greece ($n = 485$)	Norway ($n = 133$)	Greece ($n = 51$)	Scotland ($n = 121$)
1	4	5	8	9
2a	1	5	6	0
2b	5	0	8	5
4	2	22	8	36
8*	...	8	...	...
14	16	16	2	13
15	3	8	4	8
21*	...	8	...	7
Non-typable	69	28	64	22

*Not tested in this study.

Table III. Comparison of subtypes of isolates from carriers in Greece with those from carriers in northern Europe

Subtype	Percentage of isolates from			
	recruits		school children	
	Greece ($n = 489$)	Norway ($n = 133$)	Greece ($n = 51$)	Scotland ($n = 121$)
P1.1	9	5	14	3
P1.2	16	16	8	17
P1.3*	...	13	...	...
P1.4	1	0	0	0
P1.6	6	0	8	8
P1.7	6	0	4	7
P1.9	9	0	18	0
P1.10	6	0	10	0
P1.12	2	0	4	0
P1.14	2	0	0	0
P1.15	4	19	8	23
P1.16	3	8	0	4
Non-typable	36	39	26	38

*Not tested in this study.

Figure. Proportion of carriers among recruits from different regions of Greece.

population, greater Athens and East Macedonia. Over 20% of the isolates from Thessaly (22%), the Peloponnese (21%), Crete (25%) and the Aegean Islands (30%) expressed the type 14 antigen.

Isolates representative of all the 11 subtypes examined were found among those from recruits from greater Athens, East Macedonia and Crete. More isolates that did not react with the subtype antibodies were obtained from recruits from the north of Greece: Thrace, Macedonia and Epirus.

Discussion

The first two objectives of the study were to determine if the serotype and subtype reagents used for epidemiological studies in northern Europe and the Americas could be used in epidemiological studies in Greece, and if the serotype and subtype combinations associated with disease in other populations were found in Greece. The proportion of non-serotypable isolates from the Greek recruits (69%) and school children (64%) was greater than the proportion of non-serotypable isolates obtained from carriers among Norwegian recruits (28%)[6] or Scottish school children (22%). The proportion of serotype 14 isolates was similar for Greek (16%) and Norwegian (16%) recruits and Scottish school children (13%) but was much lower (2%) among Greek children. The proportion of 2b serotypes was similar for the Greek recruits and Scottish school children. Serotype 4 was the most prevalent type among the Norwegian (22%)[6] and Scottish (36%) isolates compared with 2–8% of the Greek isolates. Serotype 15 was found in 8% of isolates from north Europeans compared with 3–4% of the Greek isolates. This suggests that other serotype reagents need to be developed for epidemiological studies of meningococci from Greece and that the serotypes associated with recent outbreaks in Britain and Scandinavia are uncommon in Greece.

The proportion of isolates from the Greek recruits (36%) and school children (26%) that did not react with any of the subtype reagents was similar to that of isolates from the Norwegian recruits (39%)[6] and from Scottish school children (38%). The proportion of subtype P1.2 isolates was similar for Greek (16%) and Norwegian (16%) recruits and Scottish school children (17%) but was lower among Greek school children (8%). The proportion of subtype P1.15 was similar among the Norwegian (19%)[6] and Scottish (23%) isolates but was greater than that found in the Greek isolates (4–8%). The subtype reagents appear to discriminate between strains from Greek carriers as effectively as between carrier strains from northern Europe; however, the proportions of subtypes associated with outbreaks in northern Europe are lower.

Combinations of serotype and subtype antigens 15:P1.16, 4:P1.15 and 2a:P1.2 associated with outbreaks in northern Europe[4,5] were not found among any of the isolates in this study. Although P1.2 was the most common subtype found in the study there was only one patient isolate with this subtype, C:2b:P1.2 (table I). In contrast, the 2a:P1.2 and 2b:P1.2 combinations are found frequently among Scottish group C[12] and French serogroup B isolates and 2a:P1.2 is found frequently among French serogroup C isolates.[13] The only report of meningococcal serotypes from southeast Europe is that of the C:2a phenotype associated with disease in Italy.[14] None of the serogroup C isolates expressed this serotype.

The third objective was to determine if there were antigenic phenotypes associated with different areas of Greece, e.g., those in which there are large numbers of foreign tourists or foreign military personnel or areas such as the northeast were there is a significant Muslim population some of which might have visited Saudi Arabia; however, there was no unusual distribution of serogroups or serotypes in the different regions of Greece.

The results of the study suggest that vaccines based on serotypes and subtype combinations associated with disease in northern Europe such as the one being developed and tested in Norway will not be appropriate for Greece.

References

1. Blackwell CC, Tzanakaki G, Kremastinou J *et al.* Factors affecting carriage of *Neisseria meningitidis* among Greek military recruits. *Epidemiol Infect* 1992; **108**: 441–448.
2. Kalapothaki V, Vassilliadis P, Trichopoulos D, Kaklamani E, Sérié C. Caractéristiques séro-épidémiologiques chez les porteurs sains de Méningocoques en Grèce. *Ann Microbiol (Institut Pasteur)* 1979; **130B**: 43–54.
3. Poolman JT, Jonsdottir K, Jones DM, Lind I, Frøholm LO, Zanen HC. Meningococcal serotypes and serogroup B disease in North-West Europe. *Lancet* 1986; **2**: 555–558.
4. Poolman JT. Meningococcal vaccines. *J Med Microbiol* 1988; **26**: 170–172.
5. Abdillahi H, Poolman JT. Typing of group-B *Neisseria meningitidis* with monoclonal antibodies in whole-cell ELISA. *J Med Microbiol* 1988; **26**: 177–180.
6. Wedege E, Høiby EA, Rosenqvist ER, Frøholm LO. Serotyping and subtyping of *Neisseria meningitidis* isolates by co-agglutination, dot-blotting and ELISA. *J Med Microbiol* 1990; **31**: 195–201.
7. Blackwell CC, Weir DM, James VS *et al.* Secretor status, smoking and carriage of *Neisseria meningitidis*. *Epidemiol Infect* 1990; **104**: 203–209.
8. Blackwell CC, Tzanakaki G, Kremastinou J *et al.* Carriage of *Neisseria meningitidis* among school children in Athens. Abstracts of the 120th Meeting of the Society for General Microbiology 1991.
9. Young H. Cultural diagnosis of gonorrhoea with modified New York City (MNYC) medium. *Br J Vener Dis* 1978; **54**: 36–40.
10. Young H, Paterson IC, McDonald DR. Rapid carbohydrate utilization test for identification of *Neisseria gonorrhoeae*. *Br J Vener Dis* 1976; **52**: 172–175.
11. Abdillahi H, Poolman JT. Whole-cell ELISA for typing *Neisseria meningitidis* with monoclonal antibodies. *FEMS Microbiol Lett* 1987; **48**: 367–371.
12. Fallon RJ. Meningococcal infections in Scotland 1990. Communicable Diseases and Environmental Health in Scotland Weekly Report 1991; **25**: 91/25.4–9.
13. Riou JY, Poolman JT, Auriol J, Lamprez F, Guibourdenche M. Serosubtyping of group B, C, Y and A meningococci isolated in France in 1988. *Ann Biol Clin (Paris)* 1989; **48**: 227–231.
14. Mastrantonio P, Congiu ME, Occhionero M, Stroffolini T. Genomic fingerprints and phenotypic characteristics of strains of *Neisseria meningitidis* causing disease in Italy. *Microbiologica* 1990; **13**: 109–113.

Epidemiol. Infect. (1992), **109**, 445–452

Secretor status and humoral immune responses to *Neisseria lactamica* and *Neisseria meningitidis*

A. A. ZORGANI[1], J. STEWART[1], C. C. BLACKWELL[1], R. A. ELTON[2] AND D. M. WEIR[1]

[1] *Departments of Medical Microbiology and* [2] *Public Health Sciences, The Medical School, University of Edinburgh, Teviot Place, Edinburgh, Scotland EH8 9AG*

(*Accepted 17 June 1992*)

SUMMARY

Non-secretors of ABO blood group antigens are over-represented among patients with meningococcal diseases. Lower levels of secretory IgA reported for non-secretors have been suggested to compromise mucosal defences. Total serum and salivary IgG, IgA and IgM and levels of these isotypes specific for *Neisseria lactamica* and five isolates of meningococci were determined by ELISA for 357 pupils and staff of a secondary school in which an outbreak of meningitis occurred. There were no differences in total or specific levels of serum IgG, IgA or IgM or salivary IgG or IgA of secretors compared with non-secretors. Non-secretors had significantly lower levels of salivary IgM ($P = 0{\cdot}022$) A similar pattern was observed for levels of IgM specific for *N. lactamica* and five meningococcal isolates. The significance of these results is discussed with reference to the role of secretory IgM in protection of mucosal surfaces in infants.

INTRODUCTION

The ability of the host to resist infection or colonization by microorganisms is partly dependent on the presence of a fully functional mucosal immune system. Although IgA is the most abundant immunoglobulin in exocrine secretions such as tears, saliva and milk, IgG and IgM are also found in these fluids [1, 2]. IgM can also function as a true secretory immunoglobulin [3], reaching the mucosal surface by the identical secretory-component mediated transcellular pathway that transports IgA. The full protective potential of secretory IgA is not present in the infant at birth [4, 5]. Adult levels of secretory IgA are not reached until after 1 year of age [6, 7]. It has been suggested that in infants the presence of IgM compensates partially for the low levels of IgA [7]. IgM to poliovirus and *Escherichia coli* has been found in infants [7, 8].

Protective immunity to disease due to *Neisseria meningitidis* is associated with the presence of an intact complement system and opsonizing or bactericidal antibodies specific for the invading strain [9–11]. These antibodies can be formed through nasopharyngeal carriage of meningococci [12]. The majority of individuals

This work was presented at the 120th meeting of the Society for General Microbiology, Nottingham, UK, 27–29 August 1991.

who develop invasive disease lack protective antibody to the pathogen [9, 10]. The age range in which meningococcal infection is most prevalent (6 months to 4·5 years) reflects the natural development of antibodies to these pathogens.

Non-secretors of ABO blood group antigens are over-represented among patients with disease due to *N. meningitidis* [13, 14] and among carriers of this bacterium [15]. The lower immunoglobulin levels found in non-secretors compared with secretors have been used to explain the increased susceptibility of non-secretors to rheumatic fever and rheumatic heart disease [16, 17]. In later studies, non-secretors were reported to have lower levels of both serum [18] and salivary [19] IgA. It was suggested that specific immune responses at the mucosal surfaces of non-secretors might be compromised compared with that of secretors. The aims of the present study were to determine:

(1) if there is a difference in the amount of total serum and salivary IgA, IgG and IgM between secretors and non-secretors;
(2) if there is a difference in the levels of isotypes specific for *N. lactamica* and *N. meningitidis* in serum or saliva of secretors and non-secretors;
(3) if there is a correlation between levels of specific anti-meningococcal antibodies in secretions and in serum;
(4) if carriage of meningococci affects the levels of antibody to these bacteria found in secretors and non-secretors.

MATERIALS AND METHODS

Subjects

Sera and whole saliva specimens (357) were obtained from staff and pupils (most within the 12–18 years age group) of a school in which there was an outbreak of meningococcal disease due to a serogroup B, serotype 4, subtype P1.15 sulphonamide resistant strain (B:4:P1.15). Information regarding carriage of meningococci, secretor status, ABO and Lewis blood group antigens were reported in a previous study [15]. Informed consent to participate in the survey was obtained from parents or guardians of the children.

Isolates of neisseria

An isolate of *N. lactamica* and five different isolates of *N. meningitidis* expressing different serogroup, serotype and subtype antigens were obtained from the freeze-dried strains in the collection of the Infection and Immunity Laboratory, Medical Microbiology Department, University of Edinburgh. The outbreak strain and a B:15:P1.16 sulphonamide resistant isolate were obtained from Dr R. J. Fallon, Meningococcus Reference Laboratory (Scotland), Ruchill Hospital, Glasgow (Table 1). The bacteria were grown on Modified New York City (MNYC) agar [20] for 24 h in a humidified atmosphere with 10% CO_2.

Determination of total immunoglobulin isotype levels

The total amount of IgM and IgA were assayed by a capture ELISA method. IgG from serum and saliva was coated directly onto ELISA plates. For the measurement of total IgM and IgA, the wells of polystyrene microtitre plates were coated overnight at 4 °C with either 100 μl of mouse monoclonal anti-human IgM

Table 1. *Bacterial isolates*

Strain	Source	Serogroup	Serotype	Subtype
N. meningitidis				
A11	Patient	B	15	P1.16
A43	Patient	B	4	P1.15
A41	Carrier	B	4	—
A26	Carrier	C	4	—
A48	Carrier	NG*	4	—
N. lactamica				
L01	Carrier	NG	—	—

* Non-groupable.

Clone No. MB-11, lot 69F-4807 (1/500) (Sigma, Poole, Dorset, UK) or mouse monoclonal anti-serum IgA Clone No. GA-112, lot 99F-4803 (1/500) (Sigma) diluted in coating buffer (15 mM $NaCO_3$, 35 mM NaH_2CO_3, 3 mM NaN_3; pH 9·6). The coated plates were washed three times with phosphate buffered saline (PBS) (0·16 M-NaCl, 8 mM-NaP_2HPO_4, 1 mM-KH_2PO_4, 3 mM-KCl; pH 7·2) containing 0·01% bovine serum albumin (BSA) and Tween 20 (0·05% v/v) (PBS-Tween) and blocked with BSA (1%) in PBS (blocking buffer) for 30 min. After washing, 50 μl of serum (1/2000) or saliva (1/10) diluted in blocking buffer were added and incubated for 2 h at room temperature. Plates for detecting IgM were washed and 50 μl of sheep μ-chain-specific anti-human IgM (1/80) (Scottish Antibody Production Unit, Lanarkshire, Scotland) (SAPU) were added. After washing, 50 μl of horseradish peroxidase conjugated (HRP) donkey anti-sheep/goat IgG (1/20) were added to the plates for 1 h. For IgA plates, 50 μl HRP rabbit anti-human μ-chain-specific IgA (1/500) (Dako, High Wycombe, Bucks, UK) were added to the plates and incubated for 2 h.

Finally, the plates were washed and 50 μl of phosphate citrate buffer (0·1 M-$NaHPO_4$, 0·1 M citric acid) containing the substrate *O*-phenylene-diamine (0·4 mg/ml, pH 5·0) activated by 0·02% H_2O_2 (30% v/v). The colour was allowed to develop in the dark and the reaction was stopped after 20–30 min by adding 50 μl of H_2SO_4 (12·5%).

To determine total IgG, 100 μl of serum (1/50000) or saliva (1/2) diluted in coating buffer were added to the microtitre plates and the plates incubated overnight under the conditions used, none of the reagents was at a limiting concentration. After washing, 50 μl of HRP sheep anti-human IgG (1/20) (SAPU) diluted in blocking buffer were added for 2 h. The plates were then treated as for IgA and IgM.

Optical density (OD) at 490 nm was determined by an ELISA plate reader (Dynatech) and corrected by subtracting the OD of the corresponding blank. Samples were tested in duplicate and the readings averaged. ELISA readings were converted to mg/ml by extrapolation from the curve that was constructed from readings obtained with standard human serum: IgG = 1140 mg/dl; IgA = 250 mg/dl; IgM = 114 mg/dl (Behring lot no. 041024). A series of twofold dilutions of standard human serum (Behring, London, UK) containing known concentrations of immunoglobulin (mg/ml) were tested with the specimens from the children.

Whole cell enzyme linked immunosorbent assay

Sera and saliva were examined for anti-meningococcal antibodies by a whole bacterium ELISA. *N. meningitidis* and *N. lactamica* were cultured overnight on MNYC agar at 37 °C. Large batches of microtitre plates were coated with bacteria to minimize variations due to different antigen preparations and coating procedures. Plates were coated overnight at 4 °C with 100 μl of one of the six bacterial isolates ($6{\cdot}6 \times 10^7$ bacteria/ml). The plates were washed and blocked with PBS containing BSA (1%). The buffer was removed and plates washed with washing buffer. Undiluted serum or saliva (50 μl) was added to the wells and incubated at room temperature for 2 h. The assay was continued in the same way as ELISA for total antibodies. The assay for total and specific antibodies were determined at the same time under the same conditions.

Statistical methods

The statistical analysis of the data was performed with the package SPSS/PC+. The results were summarized by geometric means, since the logarithmic values were more normally distributed than the raw data. The significance levels for differences between groups were examined with the Mann–Whitney U test and a P value of $< 0{\cdot}05$ was regarded as significant. The association between levels of antibodies in serum and in saliva was assessed by Spearman rank correlation.

RESULTS

Total IgA, IgG and IgM antibodies of secretors and non-secretors

The specificity of the ELISA for detection of IgM, IgG and IgA was assayed with purified IgM, IgG and IgA (data not shown). There was no cross reaction between IgA, IgG and IgM. There was no marked difference in total immunoglobulin levels between sera from secretors and non-secretors. Non-secretors had higher levels of serum IgM antibody compared with secretors, but the difference was not statistically significant. There was no difference in the levels of IgA or IgG in the saliva from secretors compared with non-secretors; but, there was significantly more total IgM in the saliva of secretors ($P = 0{\cdot}0274$) (Table 2).

There was no difference in serum or salivary immunoglobulin levels of smokers (37) compared with non-smokers (320).

Specific immune responses to Neisseria *species*

There was no marked difference in antibody levels to the isolates in sera of secretors compared with non-secretors, except for IgM to the non-groupable serotype 4 isolate (Table 2).

There were significant differences in the mean specific salivary IgM immunoglobulin levels of secretors and non-secretors. Non-secretors had significantly lower levels IgM for *N. lactamica* and each of the meningococcal isolates tested (Table 2). Statistical comparison of IgA and IgG antibody levels in saliva was also performed, but there was no difference between secretors and non-secretors. For both secretors and non-secretors, the highest levels of IgM were observed for the NG:4 isolate.

Table 2. *Geometric mean levels of total and specific IgM of secretors and non-secretors*

	Non-secretors	Secretors	*P*
Serum	(*n* = 107)	(*n* = 171	
Total IgM*	1·27	1·08	0·05
Specific			
N. lactamica	9·7	7·7	0·12
B:15:P1.16	10·5	8·5	0·15
C:4	6·0	5·3	0·25
B:4	22·4	14·7	0·11
NG:4	26·8	22·2	0·03
B:4:P1.15	5·2	4·8	0·48
Saliva	(*n* = 129)	(*n* = 208)	
Total IgM	63·0	87·0	0·027
Specific			
N. lactamica	1·8	2·5	0·0000
B:15:P1.16	2·1	3·1	0·0000
C:4	2·9	3·6	0·040
B:4	2·2	3·5	0·0000
NG:4	4·7	5·9	0·017
B:4:P1.15	1·9	2·9	0·0008

* Unit for mean total serum IgM levels are expressed in mg/ml; unit for mean total and specific salivary IgM levels and specific serum IgM levels expressed in μg/ml.

Table 3. *Geometric mean of immunoglobulin levels of carriers and non-carriers*

		Mean immunoglobulin levels (μg/ml)		
		Carriers (*n*)	Non-carriers (*n*)	*P*
Serum				
Isotype strain				
IgA	C:4	18·0 (67)	13·0 (209)	0·03
IgA	B:4:P1.15	9·5 (66)	8·4 (207)	0·02
IgM	C:4	12·4 (68)	4·1 (210)	0·0000
IgM	B:4:P1.15	8·3 (66)	6·5 (207)	0·0025
IgG*	C:4	5·3 (68)	4·2 (205)	0·01
IgG	B:4:P1.15	7·2 (64)	6·1 (207)	0·0033
Saliva				
IgA	NG:4	9·7 (89)	6·6 (243)	0·01
IgM	NG:4	6·5 (89)	5·1 (243)	0·03

* Unit for mean total serum IgG are expressed in mg/ml.

The correlation between serum and salivary antibodies

Analysis by Spearman correlation test found no correlation between the levels of IgG, IgA and IgM antibodies in serum and saliva ($P > 0·05$). This suggests that the increased levels of secretory IgM found among secretors are due to locally produced immunoglobulins.

Carriage of meningococci and immunoglobulin levels

Compared with non-carriers, carriers of meningococci had significantly higher levels of salivary IgA and IgM to a NG:4 strain and serum antibodies to the

outbreak strain and to a C:4:- isolate (Table 3); however, analysis with respect to both secretor status and carriage revealed that immunoglobulin levels still differed according to secretor status when carriage was taken into account.

There was no difference in salivary immunoglobulins to the outbreak strain; but there were significantly higher levels of IgA and IgM to the NG:4 isolate.

DISCUSSION

An association between non-secretion and meningococcal disease has been reported in studies of patients in Scotland, Iceland and Nigeria [13, 14]. In this study we tested the hypothesis that there might be differences in the humoral immune responses of secretors and non-secretors that could contribute to the apparent increased susceptibility of non-secretors to meningococcal disease. Both serum and salivary immunoglobulin levels were examined.

Conflicting results have been reported for the differences in immunoglobulin levels between secretors and non-secretors; however, most of these studies measured total amounts of immunoglobulin in serum and saliva [18, 19, 21]. Lower levels of both serum [19] and salivary IgA [18] were reported for non-secretors, suggesting that specific immune responses at the mucosal surfaces of non-secretors might be reduced compared with secretors. Blackwell and her colleagues [21] did not confirm the earlier observation with single radial immunodiffusion; higher levels of IgA were associated with the presence of meningococci in the individuals from whom the saliva was obtained. There was no difference in the mean levels of total IgA in the saliva of secretors compared with non-secretors from whom no *Neisseria* spp. were isolated.

There was no difference between secretors and non-secretors in total or specific levels of salivary IgA or IgG; however, non-secretors had significantly less total IgM in their saliva compared with secretors. These differences were also observed for salivary IgM to *N. lactamica* and to five isolates of meningococci expressing different combinations of serogroup, serotype and subtype antigens. There was no correlation between levels of serum IgM and secretory IgM in saliva, suggesting that salivary IgM is locally produced and has not leaked from the serum. Although serum and saliva of carriers had significantly higher levels of antibodies to some of the neisseria isolates, the effect of secretor status on IgM remained after adjustment for the effect of carriage.

A major biological role of high molecular weight, polyvalent IgA and IgM secretory antibodies might be to provide a first line of defence against particulate and polyvalent antigens such as bacteria. Individuals with IgM deficiency appear at risk of disseminated meningococcal disease [22, 23]. It has been demonstrated that locally produced IgM in IgA-deficient patients exhibits anti-virus activity [24].

The lower levels of secretory IgM in non-secretors might contribute to susceptibility to colonization, particularly among infants under the age of 12 months in whom secretory IgM is the major class of antibody on mucosal surfaces. The presence of secretory IgM in early infancy has been suggested to compensate for the absence of secretory IgA [7]. IgM had been detected in saliva of infants who were as young as one month of age [25]. If secretory IgM provides a crucial host

defence during this period of life the lower levels of secretory IgM found for non-secretors might contribute to their apparent susceptibility to meningococcal disease. This hypothesis is under investigation at present.

ACKNOWLEDGEMENTS

A. A. Z. is supported by a grant from the General Health Laboratories, Tripoli, Libya, The work was partly funded by the Lanarkshire Health Board and TENOVUS–Scotland.

We are grateful to the students and staff of Airdrie Academy who participated in the study, to the members of the Department of Infectious Diseases and the Diagnostic Laboratory of Monklands General District Hospital and to the Lanarkshire Health Board who organized the survey.

REFERENCES

1. Brandtzaeg P, Fjellanger I, Gjeruldsen S. Human secretory immunoglobulin I. Salivary secretions from individuals with normal or low levels of serum immunoglobulins. Scand J Haemat Suppl 1970; **12**: 1–83.
2. Smith DJ, King WF, Taubman MA. Isotype, subclass and molecular size of immunoglobulins in saliva from young infants. Clin Exp Immunol 1989; **76**: 97–102.
3. Brandtzaeg P. Transport models for secretory IgA and secretory IgM. Clin Exp Immunol 1981; **44**: 221–32.
4. Haworth JC, Dilling L. Concentration of vA globulin in serum, saliva and nasopharyngeal secretions of infants and children. J Clin Lab Med 1966; **67**: 922–33.
5. Berg T. Immunoglobulin levels in infants with low birth weights. Acta Pediat Scand 1968; **57**: 369–76.
6. Burgio GR, Lanzavecchia A, Plebani A, Jayakar S, Ugazio AG. Ontogeny of secretory immunity: Levels of secretory IgA and natural antibodies in saliva. Paediatric Res 1980; **14**: 1111–4.
7. Mellander L, Carlsson B, Hansson LA. Appearance of secretory IgM and IgA antibodies to *Escherichia coli* in saliva during early infancy and childhood. J Pediat 1984; **104**: 564–8.
8. Mellander L, Carlsson B, Hanson LA. Secretory IgA and IgM to *E. coli* and poliovirus type 1 antigens occur in amniotic fluid, meconium and saliva from newborns. A neonatal immune response without antigenic exposure: a result of idiotypic induction? Clin Exp Immunol 1986; **63**: 555–61.
9. Goldschneider I, Gotschlich EC, Artenstein MS. Human immunity to the meningococcus. I. The role of humoral antibody. J Exp Med 1969; **129**: 1307–26.
10. Goldschneider I, Gotschlich EC, Artenstein MS. Human immunity to the meningococcus. II. Development of natural immunity. J Exp Med 1969; **129**: 1327–48.
11. Lee TJ, Ustanger PD, Snyderman R, Young WJ, Sparling PF. Familial deficiency of the seventh component of complement associated with recurrent bacteraemic infection due to Neisseria. J Infect Dis 1978; **138**: 359–68.
12. Reller LB, MacGregor RR, Beaty HN. Bactericidal antibody after colonization with *Neisseria meningitidis*. J Infect Dis 1973; **127**: 56–62.
13. Blackwell CC, Jónsdottir K, Hanson M, et al. Non-secretion of ABO blood group antigens predisposing to infection by *Neisseria meningitidis* and *Streptococcus pneumoniae*. Lancet 1986; ii: 284–5.
14. Blackwell CC, Jónsdottir K, Mohammed I, Weir DM. Non-secretion of blood group antigens. A genetic factor predisposing to infection by *N. meningitidis*. In: Gonococci and meningococci, Poolman JT, et al., eds. Dordrecht: Kluwer Academic Publishers, 1988; 633–6.
15. Blackwell CC, Weir DM, James VS, et al. Secretor status, smoking and carriage of *Neisseria meningitidis*. Epidemiol Infect 1990; **104**: 203–9.
16. Glynn AA, Glynn LE, Holborow EJ. Secretor status of rheumatic-fever patients. Lancet 1956; ii: 759–62.

17. Clarke CA, McConnell RB, Sheppard PM. ABO blood groups and secretor character in rheumatic carditis. Br Med J 1960; **1**: 21–3.
18. Grundbacher FJ. Immunoglobulins, secretor status and the incidence of rheumatic fever and rheumatic heart disease. Hum Hered 1972; **22**: 399–404.
19. Waissbluth JG, Langman MS. ABO blood groups, secretor status, salivary proteins, serum and salivary immunoglobulin concentrations. Gut 1971; **12**: 646–9.
20. Young H. Cultural diagnosis of gonorrhoea with Modified New York City (MNYC) medium. Br J Vener Dis 1978; **54**: 36–40.
21. Blackwell CC, Jónsdottir K, Weir DM, et al. Blood group, secretor status and susceptibility to bacterial meningitis. FEMS Microbiol Immunol 1989; **47**: 351–6.
22. Hobbs JR, Milner RDG, Watt PJ. Gamma-M deficiency predisposing to meningococcal septicaemia. Br Med J 1967; **4**: 583–6.
23. Jones DM, Tobin BM, Butterworth A. Three cases of meningococcal infection in a family, associated with a deficient immune response. Arch Dis Child 1973; **48**: 742–3.
24. Ogra PL, Coppola PR, MacGillivray MH, Dzierba JL. Mechanism of mucosal immunity to viral infections in A immunoglobulin-deficiency syndrome (37900). Proc Soc Exp Biol Med 1974; **145**: 811–6.
25. Gleeson M, Cripps AW, Clancy RL, Husband AJ, Hensley MJ, Leeder SR. Ontogeny of secretory immune system in man. Aus NZ J Med 1982; **12**: 255–8.

Epidemiol. Infect. (1992), **109**, 507–518

Factors influencing oral carriage of yeasts among individuals with diabetes mellitus

F. Z. ALY, C. C. BLACKWELL, D. A. C. MACKENZIE, D. M. WEIR AND B. F. CLARKE

Department of Medical Microbiology, University of Edinburgh, Medical School Building, Teviot Place, Edinburgh EH8 9AG

(*Accepted 8 June 1992*)

SUMMARY

A total of 439 individuals with diabetes mellitus were examined for carriage of yeasts by the oral rinse and palatal swab techniques. Eighteen genetic or environment variables were assessed for their contribution to carriage of yeasts. The factor contributing to palatal and oral carriage of yeasts among individuals with insulin dependent diabetes mellitus (IDDM) was age ($P < 0{\cdot}01$). The factor contributing to palatal carriage of yeasts among individuals with non-insulin dependent diabetes mellitus (NIDDM) was poor glycaemic control (glycosuria $P < 0{\cdot}01$); carriage in the oral cavity as a whole was influenced additionally by non-secretion of ABH blood group antigens ($P < 0.05$). Introduction of a denture altered the above risk factors. For individuals with IDDM, oral carriage was associated with the presence of retinopathy ($P < 0{\cdot}05$); palatal carriage was influenced by poor glycaemic control (HbA_1 $P < 0{\cdot}01$, plasma glucose levels $P < 0{\cdot}05$) and age ($P < 0{\cdot}05$). For those with NIDDM, palatal carriage was associated with continuous presence of the denture in the mouth ($P < 0{\cdot}01$); oral carriage was associated with plasma glucose levels ($P < 0{\cdot}05$).

INTRODUCTION

Oral candidiasis is most prevalent as chronic atrophic candidiasis [1]. Individuals with diabetes are prone to infection; and chronic atrophic candidiasis has been reported to be more prevalent among diabetic individuals compared with non-diabetic controls [2]. Since disease is frequently preceded by colonization, it is important to identify factors which predispose to colonization. Few studies have examined the factors which predispose to colonization of diabetic patients by *Candida albicans*; and none of these specifically analysed patients with insulin dependent diabetes mellitus (IDDM) separately from those with non-insulin dependent diabetes mellitus (NIDDM) [2–6].

Both genetic [7, 8] and environmental factors [9] have been reported to affect carriage of *Candida* species. Group A β-haemolytic *Streptococcus pyogenes* are isolated more frequently from the pharynx of individuals who are non-secretors of ABH antigens [10] and non-secretors were significantly over-represented among carriers of meningococci [11]. Among non-diabetic individuals and individuals

with NIDDM non-secretors of blood group O are over-represented among carriers of *C. albicans* compared with secretors [7, 8].

Environmental factors variably reported to be associated with carriage of yeasts include: presence of a denture; continuous wearing of a denture; denture fit, occlusion, trauma, hygiene and age; smoking; and control of diabetes [3, 12].

This was the first study designed to compare the factors contributing to carriage of yeasts in individuals with IDDM or NIDDM; its aims were:

(1) To compare the oral rinse technique with the palate swab method for isolating yeasts.
(2) To compare the species of yeasts isolated from patients with IDDM or NIDDM.
(3) To assess the association between secretor status and carriage taking into account denture status.
(4) To dissect the contributions of the following variables to the carriage of yeasts and, specifically, *C. albicans*: age; sex; type of diabetes; control of diabetes as measured by glycosylated haemoglobin A_1 (HbA_1), random plasma glucose levels, persistent glycosuria and albuminuria; diabetic complications – retinopathy, neuropathy, nephropathy; antibiotic usage; corticosteroid treatment; smoking; alcohol consumption; presence of the denture in the mouth at night; denture fit, extension, occlusion hygiene and age; presence of denture stomatitis; and history of superficial candida infections.

MATERIALS AND METHODS

Subjects

A total of 439 subjects attending for routine follow up examination at the diabetic out-patient clinic, Royal Infirmary, Edinburgh were sampled. An initial pilot study examined 80 individuals and was followed by a study that sampled 359 individuals between September 1988 and March 1989. The method of selection was stratified random selection according to sex and type of diabetes.

Clinical history

Each subject was classified as insulin dependent (IDDM) or non-insulin dependent (NIDDM) according to family history of diabetes, clinical history of onset, requirement for insulin, progression of the disease. Three of the 439 subjects sampled could not be classified.

A full medical history including the presence of diabetic complications (retinopathy, neuropathy and nephropathy) was obtained during interview and from the patients' records. A history of medications, with particular reference to antibiotics or corticosteroid-containing preparations, within the past 6 months was noted. A social history of alcohol consumption and smoking was recorded. Subjects were questioned about history of superficial infections due to candida. Glycosuria and albuminuria were recorded as persistent if subjects had positive urine samples on more than two consecutive appointments at the clinic. None of the subjects used any oral preparations containing antiseptics within the previous 6 months.

Clinical examination

A thorough oral examination of both soft and hard tissues was carried out. The occlusion, fit, extension and hygiene of a denture where present was recorded as 'good' or 'poor'. The age of the denture was recorded as well as whether it was left out of the mouth at night.

Samples

Venous blood was obtained for ABO blood grouping and Lewis antigen determination. Routine analyses for glycosylated haemoglobin (HbA_1) and random plasma glucose were recorded. Swabs were obtained from five sites (palate, tongue, floor, right and left angles of the mouth) and inoculated immediately into malt broth. Each subject provided a fresh, unstimulated sample of saliva which was collected in a sterile Universal container.

Subjects were requested to rinse with 10 ml of sterile phosphate buffered saline (PBS) for 1 min and to return the contents to a sterile Universal container.

Laboratory analysis

ABO blood group was determined by slide agglutination with monoclonal anti-A and anti-B antibodies (Scottish National Blood Transfusion Service). Secretor status was determined by the presence of Lewis antigen on red blood cells by tube agglutination with monoclonal anti-Le^a and anti-Le^b antibodies (Scottish National Blood Transfusion Service). The haemagglutination inhibition method with boiled saliva was used to confirm the Lewis antigen results for 159 individuals [13].

The Corning electrophoresis method was used for measuring HbA_1 (normal range 4·5–8·0%, coefficient of variance = 4%).

The swabs in malt broth were incubated at 37 °C for 36–48 h to enrich for *C. albicans*, plated onto malt agar and incubated for a further 36–48 h.

The mouth rinse was concentrated by centrifugation and resuspended in 1 ml of PBS; 20 μl of the suspension were inoculated onto malt agar plates and incubated at 37 °C for 36–48 h. The number of colonies per sample were recorded.

Pure colonies were subcultured and identified with the API 20C Auxanogram (API Systems S.A., France). All were also identified by the conventional methods of germ-tube production in horse serum, urease test and hyphae production on corn-meal agar following incubation at 28 °C for 48 h [14].

Statistical analysis

All results were coded and a computerized database was set up to facilitate analysis by the SPSSX. Univariate analysis was by the χ^2 (with Yates' correction) or Wilcoxon rank sum tests. MacNemar's test for paired alternatives was used to compare the results of the oral rinse and the palate swab.

Stepwise linear discriminant analysis (Wilk's method) was used to identify which combinations of factors best predicted yeast carriage. During the analysis, predictor variables can be added or removed in a stepwise manner, each variable being selected on the basis of an F level of 4 for entry or removal, corresponding to $P = 0{\cdot}05$.

RESULTS

Table 1 summarizes the characteristics of the population sampled with respect to their diabetic status. Individuals with IDDM were younger, had a higher mean value of HbA_1; and they had a higher proportion of non-secretors (36·4%) compared with individuals with NIDDM (22%) ($\chi^2 = 10{\cdot}15$; $P < 0{\cdot}005$). The mean number of cigarettes per day smoked by subjects with IDDM or NIDDM were similar.

Mycological profile of the sample population

Only 29% (128/439) of the individuals examined had no yeasts in any of the five sites swabbed; this compared well with the oral rinse technique in which 34% of the individuals were culture negative. The concordance between the results obtained by these methods was 85%. By MacNemar's test for paired alternatives, there was no significant difference between the discordant pairs obtained by the two sampling methods ($P > 0{\cdot}1$). Table 2 compares the species of yeasts cultured from swabs of the five sites of the mouth with those obtained from the oral rinse from individuals with IDDM or NIDDM.

Univariate analysis of factors affecting carriage

Tables 3–8 present analysis of factors examined for their association with carriage of yeasts; similar results were obtained when carriage of *C. albicans* was analysed separately.

Isolation of yeasts from the palate or from the oral rinse was not associated with secretor status when the results were analysed by type of diabetes and/or denture status.

Blood group was not associated with palatal carriage. When carriage was assessed by the oral rinse, however, individuals with NIDDM who wore dentures and who were of O blood group were more likely to be carriers than those of blood group A ($\chi^2 = 7{\cdot}93$, $P = 0{\cdot}05$) (Table 3). Comparisons with B and AB were not done because of the small numbers of individuals with these blood groups. Analysis of secretor status with reference to blood group revealed a significant association between non-secretion and increased frequency of carriage of yeasts among individuals with IDDM who were blood group A and who wore dentures; there were 9/19 A-secretor carriers compared with 12/12 A-non-secretor carriers ($\chi^2 = 7{\cdot}07$, $P = 0{\cdot}008$).

Yeasts were isolated significantly more frequently from the palate of patients with IDDM with or without dentures (Table 4). Similar results were obtained by the oral rinse method.

Increase in age was significantly associated with a decreased frequency of isolation of yeasts from the palate of patients with IDDM but not those with NIDDM (Table 5). Similar results were obtained by the oral rinse technique.

The HbA_1 values of palatal carriers were higher compared with non-carriers; particularly among individuals with IDDM (Table 6). Results from the oral rinse technique showed a similar trend but did not attain significance at $P < 0{\cdot}05$ for individuals with IDDM or NIDDM.

The mean number of cigarettes per day was a highly significant factor

Table 1. *Characteristics of the sample population*

Type of diabetes	Age mean (S.D.)	HbA_1 mean (S.D.)	Smokers (%)	Cigarettes per day	Non-secretors (%)	Secretors (%)
IDDM (n = 231)	40·3 (16·0)	10·3 (2·1)	41	17·3 (11·1)	36·4	63·6
NIDDM (n = 205)	58·1 (9·3)	9·8 (2·3)	34	17·3 (11·7)	22·0	78·0

Table 2. *Species of yeast isolated from individuals with IDDM or NIDDM with or without dentures (percentages)*

	Patient category	Sample	None	*C. albicans*	*C. tropicalis*	Other
No dentures						
IDDM	Oral rinse	33	47	2	0	18
(n = 150)	Palate	44	48	1	1	6
	Tongue	38	54	2	0	6
	Floor of mouth	41	54	1	1	3
	Right angle	56	38	0	0	5
	Left angle	60	35	0	0	5
NIDDM	Oral rinse	40	28	2	0	31
(n = 70)	Palate	63	28	0	0	9
	Tongue	63	32	0	0	5
	Floor of mouth	70	27	0	0	3
	Right angle	68	27	0	0	5
	Left angle	75	24	0	0	2
Dentures						
IDDM	Oral rinse	24	43	4	0	29
(n = 79)	Palate	33	47	9	2	9
	Tongue	37	49	5	2	8
	Floor of mouth	34	49	5	3	8
	Right angle	50	29	9	1	10
	Left angle	42	41	6	2	9
NIDDM	Oral rinse	41	24	6	3	27
(n = 135)	Palate	51	29	6	3	12
	Tongue	51	34	8	2	6
	Floor of mouth	53	31	7	3	7
	Right angle	54	25	9	3	9
	Left angle	58	24	10	3	6

Table 3. *Blood groups and carriage of yeasts assessed by the oral rinse technique*

Category	Blood group	Non-carriers no. (%)	Carriers no. (%)	χ^2	P
IDDM	O	22 (39)	34 (61)	1·49	0·22
No dentures	A	11 (26)	32 (74)		
IDDM	O	5 (16)	26 (84)	1·24	0·27
With dentures	A	10 (31)	22 (69)		
NIDDM	O	9 (33)	18 (67)	0·13	0·73
No dentures	A	7 (44)	9 (56)		
NIDDM	O	17 (32)	36 (68)	7·93	0·005
With dentures	A	20 (67)	10 (33)		

Table 4. *Type of diabetes and isolation of yeasts from the palatal swab*

Category	Non-carriers no. (%)	Carriers* no. (%)	χ^2	P†
All				
IDDM	70 (39)	108 (61)	8·5	0·004
NIDDM	101 (55)	82 (45)		
Without dentures				
IDDM	48 (44)	62 (56)	5·03	0·014
NIDDM	40 (62)	24 (38)		
With dentures				
IDDM	22 (33)	44 (67)	4·81	0·013
NIDDM	61 (51)	58 (49)		

* All yeasts including *C. albicans*.
† *P* value refers to comparisons between IDDM and NIDDM

Table 5. *Median age and carriage of yeasts determined by palate swab*

Category	Median age (years) Non-carriers	Carriers	P*
IDDM with dentures	58·00	49·00	0·033
(*n*)	(22)	(44)	
IDDM without dentures	38·50	29·00	0·011
(*n*)	(48)	(62)	
NIDDM with dentures	61·00	59·00	0·07
(*n*)	(61)	(58)	
NIDDM without dentures	56·00	50·50	0·09
(*n*)	(39)	(24)	

* *P* determined by Mann–Whitney test.

Table 6. *Glycaemic control (HbA_1) and carriage determined by palate swab*

Category	Median HbA_1 units Non-carriers	Carriers	P*
IDDM with dentures	9·55	11·10	0·067
(*n*)	(18)	(236)	
IDDM without dentures	9·30	10·60	0·017
(*n*)	(45)	(55)	
NIDDM with dentures	9·30	9·50	0·15
(*n*)	(57)	(52)	
NIDDM without dentures	8·70	9·90	0·26
(*n*)	(40)	(21)	

* *P* determined by Mann–Whitney test.

Table 7. *Smoking and carriage of yeasts determined by palate swab*

Category	Mean cigarettes/day: Non-carriers	Carriers	P*
All	4·54	7·83	< 0·001
(*n*)	(156)	(181)	
IDDM with dentures	7·95	9·20	0·24
(*n*)	(20)	(44)	
IDDM without dentures	5·72	7·32	0·43
(*n*)	(43)	(59)	
NIDDM with dentures	2·89	6·87	0·051
(*n*)	(57)	(54)	
NIDDM without dentures	4·28	9·09	0·13
(*n*)	(36)	(23)	

* P determined by Mann–Whitney test.

Table 8. *Continuous wearing of denture and isolation of yeasts from the palate*

Category	Denture presence	Non-carriers no. (%)	Carriers no. (%)	χ^2	P
All	Yes	30 (40)	45 (60)	6·61	0·01
	No	32 (65)	17 (35)		
IDDM	Yes	10 (32)	21 (68)	0·002	0·96
	No	5 (38)	8 (62)		
NIDDM	Yes	20 (45)	24 (55)	5·97	0·015
	No	27 (75)	9 (25)		

associated with carriage of the whole population sampled (Mann–Whitney $P < 0·001$). When results were analysed with respect to types of diabetes and denture status, smoking was of marginal significance ($P = 0·051$) only among denture wearers with NIDDM (Table 7).

Yeasts were isolated more often from the palate of subjects who left their dentures in the mouth at night; however, this was observed only among individuals with NIDDM (Table 8). This pattern was not found when carriage was assessed by the oral rinse method.

The following variables were not associated with frequency of carriage of yeasts by either isolation technique; sex; duration of diabetes; complications – neuropathy, nepthropathy and retinopathy; alcohol consumption; contraceptive pill; systemic corticosteroid treatment; topical corticosteroid application; antibiotics; fit, occlusion or hygiene of denture; persistent albuminuria; or history of superficial candida infections. Denture status was not significantly associated with frequency or density of colonization by yeasts.

Multivariate analysis

Univariate analysis might incorporate dependent variables which need prior knowledge to control for their effect. The multivariate analysis identified factors

Table 9. *Palatal carriage of yeasts: variables isolated by multivariate analysis (Wilk's Method)*

Diabetes	Dentures	Variables isolated	% cases correctly predicted
IDDM ($n = 110$)	No	Age ($P < 0{\cdot}05$)	61
IDDM ($n = 51$)	Yes	HbA_1 ($P < 0{\cdot}01$) Age ($P < 0{\cdot}05$) Plasma glucose ($P < 0{\cdot}05$)	71
NIDDM ($n = 49$)	No	Glycosuria ($P < 0{\cdot}01$)	78
NIDDM ($n = 80$)	Yes	Continuous wear of denture ($P < 0.01$)	64

Table 10. *Oral carriage of yeasts: variables isolated by the multivariate analysis (Wilk's Method)*

Diabetes	Dentures	Variables isolated	% cases correctly predicted
IDDM ($n = 97$)	No	Age ($P < 0{\cdot}01$)	68
IDDM ($n = 71$)	Yes	Retinopathy ($P < 0{\cdot}05$)	76
NIDDM ($n = 43$)	No	Glycosuria ($P < 0{\cdot}01$) plasma glucose ($P < 0{\cdot}05$) Non-secretion ($P < 0{\cdot}05$)	67
NIDDM ($n = 78$)	Yes	Plasma glucose ($P < 0{\cdot}05$)	48

which contribute to carriage of yeasts among individuals with IDDM or NIDDM who wear dentures and those without dentures. Tables 9 and 10 summarize the results obtained from the palate swab and oral rinse techniques respectively. Similar results were obtained when carriage of *C. albicans* was analysed separately. The percentage of carriers correctly predicted by the isolated variables indicates their prognostic value.

Individuals with IDDM who did not wear dentures were best segregated on the basis of their age into non-carriers and carriers of yeasts; carriage decreased with increased age. This was true when carriage was assessed by either technique. The univariate analysis of this group of patients showed similar associations; younger individuals were more prone to carriage of yeasts.

Among individuals with IDDM who wore dentures, an increase in the frequency of palatal carriage of yeasts was associated with an increase in HbA_1 level ($P < 0{\cdot}01$); younger individuals ($P < 0{\cdot}05$) and increased random plasma glucose levels ($P < 0{\cdot}05$). The variable which was the most efficient predictor of carriage assessed by the oral rinse technique was, however, the presence of retinopathy ($P < 0{\cdot}05$). Univariate analysis of this group of patients showed age to be associated with palatal carriage.

Individuals with NIDDM who did not wear dentures were more likely to be palatal carriers of yeasts if they had persistent glycosuria ($P < 0{\cdot}05$). Carriage

assessed by the oral rinse method was influenced by persistent glycosuria ($P < 0{\cdot}01$), higher random plasma glucose levels ($P < 0{\cdot}05$) and non-secretion of blood group antigens ($P = 0{\cdot}05$). Univariate analysis of this group revealed an association between persistent glycosuria and carriage among individuals with NIDDM ($\chi^2 = 5{\cdot}32$, $P = 0{\cdot}02$).

Individuals with NIDDM who wore dentures were at risk of palatal carriage of yeasts if they wore their dentures continuously ($P = 0{\cdot}01$). Carriage of yeasts assessed by the oral rinse technique was best predicted by an increased random plasma glucose levels ($P < 0{\cdot}05$). Comparison with univariate analysis showed that continuous wearing of dentures was a significant factor only when carriage was assessed by palatal swab.

DISCUSSION

The results are discussed in the context of the objectives of the study. The first objective of the study was to compare isolation of yeasts by the oral rinse technique with those obtained by the palate swab. The oral rinse technique compared well with the results of the swab technique; there was 85% concordance between the results obtained with the two methods.

C. albicans was the species most frequently isolated from the swabs while species other than *C. albicans* were isolated more often from the oral rinse. This might be due to incubation of swabs in the malt broth suppressing other species of yeasts. By the swab technique, yeasts were isolated most frequently from the tongue followed by the palate, floor of the mouth and the angles of the mouth. Similar results have been reported [3]. This supports the suggestion that the tongue might act as a reservoir for yeasts [15]. For future work, the technique appropriate for the objectives of the study should be employed. The oral rinse technique can be used where quantitative and overall carriage of yeasts are required; swabs can be used for examination of specific sites for carriage.

In this study, 66% of the diabetics were carriers of yeasts by the oral rinse technique, a figure slightly higher than those reported in previous studies of diabetics (41–62%) [2, 3, 5, 6, 16].

The second objective was to compare isolation of yeasts from patients with IDDM compared with those with NIDDM. *C. glabrata* and *C. tropicalis* were isolated more frequently from patients with either IDDM or NIDDM who wore dentures. There were significantly more carriers among individuals with IDDM compared with NIDDM subjects; no other study has found this association [2, 3, 16]. This might be due to the smaller sample sizes of other studies, differences in populations sampled and their distinction of IDDM from NIDDM based solely on treatment of the diabetic condition. In this study subjects were classified as IDDM or NIDDM according to family history of diabetes, clinical history of onset, requirement for insulin and progression of the disease.

By the API identification system, some studies have found 97% of isolates to be *C. albicans* [5, 17]. In this study only 54% of the isolates were *C. albicans* (Table 2); this figure includes the proportion that was not identified by API 20C Aux as *C. albicans* but found to be so by conventional methods. These results are similar to studies which found 60% of isolates from diabetics to be *C. albicans* [3, 16]. This

emphasizes the need for accurate identification as patients with diabetes are more likely to carry and to have disease due to species other than *C. albicans* [18] which might not be sensitive to routinely prescribed oral antifungal agents [19].

The third objective was to assess the association between secretor status and carriage of yeasts among individuals with IDDM separately from those with NIDDM. In most diseases the influence of blood group or secretor status is marginal; sample sizes need to be large enough to discern such relationships with confidence [20]. By univariate analysis, other studies found a relationship between non-secretion of blood group antigens and carriage of yeasts among diabetics [8, 17]. These studies did not take into account the denture status [8] or type of diabetes [17] of the sample population. Another study did not report an association between secretor status and carriage of yeasts or development of disease [2]; however, the number of subjects was much smaller, denture status and type of diabetes were not considered.

Among individuals with NIDDM, the results of the multivariate analysis show that non-secretion of blood group antigens was a marginally significant factor influencing carriage in the oral cavity as a whole. Palatal carriage of yeasts was not dependent on secretor status. Density of colonization reflected in the quantitative carriage of yeasts assessed by the oral rinse is influenced by secretor status; carriage *per se* does not depend on secretor status.

The fourth objective of the study was to dissect the contribution of various other factors to carriage of yeasts. The following factors were not associated with carriage by either univariate or multivariate analyses: sex; duration of diabetes; diabetic complications of neuropathy or nephropathy; alcohol consumption; contraceptive pill; systemic corticosteroid treatment; topical corticosteroid application; antibiotic usage; fit, occlusion or hygiene of denture; persistent albuminuria; or history of superficial candida infections. Those for which significant associations were found are discussed below with reference to previous reports.

The prevalence of yeasts in the adult mouth has been shown to rise with age. The effects of age are not always easily separated from disease and medical treatment [21, 22]. Age was not related to isolation of yeasts from diabetics [2, 3, 17]. In a Canadian study [6], diabetics over 50 years of age had a higher density of yeasts compared with those less than 50 years old. Age was not isolated as a significant factor in the multivariate analysis, and the authors suggested that carriage in the older age group might be due to the higher number of denture wearers. In the study reported here, increase in age was associated with a decrease in the frequency of carriage especially among non-denture wearers with IDDM (Tables 5 and 9). This was unexpected; one explanation might be that decreased isolation of yeasts with increasing age reflects the efficiency of the mucosal immune defences against yeasts with increased frequency of challenge. Studies on vaginal carriage of yeasts found an inverse correlation between isolation of yeasts and levels of serum anti-candida IgA [23]. Secretory anti-candida IgA has been reported to correlate with levels of serum IgA [24]. Analysis of secretory and humoral immune responses of the study population to yeasts are needed to obtain evidence for this hypothesis.

Poor glycaemic control (HbA_1 and plasma glucose) was a significant factor

associated with palatal carriage of yeasts among patients with IDDM who wore dentures. Carriage assessed by the oral rinse was not associated with HbA_1 levels; similar results were reported in other studies [2, 5]. The single study in which multivariate analysis was used found an association between HbA_1 level and colonization determined by palate or denture base swab; however, this study did not differentiate individuals with IDDM from those with NIDDM [6]. Further evidence that diabetic control is associated with carriage was the significant association between carriage and plasma glucose levels or persistent glycosuria among patients with NIDDM who did not wear dentures. Studies on other diabetic populations reported similar results [16], but the majority did not find this association [2, 3, 6, 17].

Retinopathy was of marginal significance as a predictor of carriage among denture wearers with IDDM. This complication results from microvascular changes associated with diabetes which might also impair immune response through inadequate diffusion of tissue mediators and indicates that systemic changes associated with diabetes predispose to colonization.

Studies in non-diabetic populations reported a positive correlation between smoking and carriage of bacteria [25, 26] and yeasts [27]. Among diabetics, smoking was a significant factor influencing carriage of yeasts; however, the type of diabetes and denture status were not taken into account [3]. Although similar association was found in this study, this association was not significant when results were analysed with respect to type of diabetes and denture status.

In contrast to other studies which reported a higher prevalence and/or density of yeasts among diabetics who wore dentures [2, 3, 5, 6], we found no correlation with frequency or quantity of yeasts isolated with either technique. Similar results were reported by Darwazeh and colleagues [17]. There is no obvious explanation for these discrepancies. Continuous presence of the denture might influence analysis of denture status results; however, we found this association only for palatal carriage among patients with NIDDM (Tables 8 and 9). Other studies found continuous presence of dentures associated with increased frequency and density of yeasts [3, 17].

The contribution of factors such as antibiotics and corticosteroids to carriage of yeasts is not easily differentiated from that of the underlying illness. Most of the studies in which associations between carriage and these treatments were found were carried out on hospitalized patients. The present study examined out-patients, the majority of whom were not on either of these treatments; therefore, no inferences can be made on the role of these therapies on oral carriage.

REFERENCES

1. Odds FC. Factors that predispose the host to candidiasis. In: Candida and candidiasis: a review and bibliography. 2nd edn. Baillière Tindall. 1988: 93–114.
2. Lamey PJ, Darwazeh A, Fisher BM, Samaranayake LP, McFarlane TW, Frier BM. Secretor status, candidal carriage and candidal infection in patients with diabetes mellitus. J Oral Pathol 1988; **17**: 354–7.
3. Tapper-Jones LM, Aldred MJ, Walker DM, Hayes TM. Candidal infections and populations of *Candida albicans* in mouths of diabetics. J Clin Pathol 1981: **34**: 706–11.
4. Barlow AJE, Chattaway FW. Observations on the carriage of *C. albicans* in man. Br J Dermatol 1969; **81**: 103–6.

5. Fisher BM, Lamey P-J, Samanayake LP, MacFarlane TW. Carriage of *Candida* species in the oral cavity in diabetic patients: relationship to glycaemic control. J Oral Pathol 1987; **16**: 282–4.
6. Hill LVH, Tan MH, Pereira LH, Embil JA. Associations of oral candidiadis with diabetic control. J Clin Pathol 1989; **42**: 502–5.
7. Burford-Mason AP, Welsby JCP, Willoughby JMT. Oral carriage of *C. albicans*, ABO blood group and secretor status in healthy individuals. J Med Veter Mycol 1988; **26**: 49–56.
8. Blackwell CC, Aly FZM, James VS, et al. Blood group, secretor status and oral carriage of yeasts among patients with diabetes mellitus. Diabetic Res 1989; **12**: 101–4.
9. Ryley JF. Review article: Pathogenicity of *Candida albicans* with particular reference to the vagina. J Med Vet Mycol 1986; **24**: 5–22.
10. Haverkorn MJ, Goslings WRO. Streptococci, ABO blood groups and secretor status. Am J Hum Genet 1969; **21**: 360–75.
11. Blackwell CC. Genetic susceptibility to infectious agents. Proc Roy Coll Phys Edin 1989; **19**: 129–35.
12. Budtz-Jorgensen E, Bertram V. Denture stomatitis. I. The etiology in relation to trauma and infection. Acta Odontol Scand 1970; **28**: 71–92.
13. Mollison PL. Blood transfusion in clinical medicine, 6th ed. Oxford: Blackwell Scientific Publications, 1976; 414–82.
14. Milne LJR. Fungi. In: Practical medical microbiology, 13th ed. Edinburgh: Churchill Livingstone, 1988; 675–99.
15. Soammes JV, Southam JC. Infections of the oral mucosa. In: Oral pathology. Oxford: Oxford University Press, 1985: 153–8.
16. Odds FC, Evans EGV, Taylor MAR, Wales JK. Prevalence of pathogenic yeasts and humoral antibodies to *Candida* in diabetic patients. J Clin Pathol 1978; **31**: 840–4.
17. Darwazeh AMG, Lamey PL, Samaranayake LP, et al. The relationship between colonization, secretor status and *in vitro* adhesion of *Candida albicans* to buccal epithelial cells from diabetics. J Med Microbiol 1990; **33**: 43–9.
18. Aly FZM, Blackwell CC, Mackenzie DAC, et al. Chronic atrophic oral candidiasis among patients with diabetes mellitus – role of secretor status. Epidemiol Infect 1991; **106**: 355–63.
19. Dick JD, Nerz WG, Saral L. Incidence of polyene-resistant yeasts recovered from clinical specimens. Antimicrob Agents Chemother 1980; **18**: 158–63.
20. Mourant AE, Kopec AC, Domaniewska-Sobczak K. Blood groups and diseases. Oxford: Oxford University Press, 1978.
21. Marples MJ. Microbiological studies in Western Samoa. II The isolation of yeast-like organisms from the mouth with a note of some dermatophytes isolated from lesions of *Tinea corporis*. Trans Roy Soc Trop Med Hyg 1960; **54**: 166–70.
22. Smits BJ, Prior AP, Alabaster PG. Incidence of *Candida* in hospital in-patients and the effect of antibiotic therapy. Br Med J 1966; **1**: 208–10.
23. Schonheyder H, Melbye M, Biggar RJ, Ebbesen P, Neuland CY, Stenderup A. Oral yeast flora and *Candida albicans* in homosexual men. Mykosen 1984; **27**: 539–44.
24. Warnock DW, Hilton AL. Value of the indirect immunofluorescence test in the diagnosis of vaginal candidiasis. Br J Vener Dis 1976; **52**: 187–9.
25. Blackwell CC, Weir DM, James VS et al. Secretor status, smoking and carriage of *Neisseria meningitidis*. Epidemiol Infect 1990; **104**: 203–9.
26. Blackwell CC, Tzanakaki G, Kremastinou J, et al. Factors affecting carriage of *Neisseria meningitidis* among Greek military recruits. Epidemiol Infect. In press.
27. Arendorf TM, Walker DM, Kingdom RJ, Rous JRS, Newcombe RG. Tobacco smoking and denture wearing in oral candidal leukoplakia. Br Dent J 1983; **155**: 340–3.

EFFECT OF RESPIRATORY SYNCYTIAL VIRUS INFECTION ON BINDING OF *NEISSERIA MENINGITIDIS* AND *HAEMOPHILUS INFLUENZAE* TYPE B TO A HUMAN EPITHELIAL CELL LINE (HEP-2)

M.W. RAZA[1], M.M. OGILVIE[1], C.C. BLACKWELL*[1], J. STEWART[1], R.A. ELTON[2] and D.M. WEIR

[1]Department of Medical Microbiology and [2]Medical Statistics Unit, Department of Public Health Sciences, University of Edinburgh Medical School, Teviot Place, Edinburgh EH8 9AG

Running head: Bacterial binding to RSV-infected cells.

* Corresponding author

ref: ccb/mc/c/rsv-inf2

SUMMARY

It has been suggested that individuals might be more readily colonized with bacteria that cause meningitis through enhanced binding of the bacteria to virus-infected epithelial cells. As respiratory syncytial virus (RSV) affects infants and children in the age group also susceptible to bacterial meningitis, we tested the hypothesis that infection of HEp-2 cells by RSV might enhance binding of Neisseria meningitidis or Haemophilus influenzae type b (Hib). Attachment of fluorescein-labelled bacteria to HEp-2 cells was measured by flow cytometry, and RSV-infected cells bound significantly more meningococci ($p < 0.001$) and Hib ($p < 0.01$) than uninfected cells. Although the isolates expressed different antigenic characteristics (3 meningococci and 5 Hib), all showed a similar pattern of binding. The results are discussed with reference to the methods used for detection of bacterial binding and to interactions that might explain the increased binding to RSV-infected cells.

INTRODUCTION

Clinical, epidemiological and experimental evidence indicates that virus infections can be predisposing factors for invasive bacterial diseases and carriage of potentially pathogenic bacteria [1-6]. Factors considered to contribute to this effect include: immune suppression [7-9]; diminished phagocytosis by polymorphonuclear leucocytes [10], local oedema formation and tissue injury; loss of mucociliary function and decreased bacterial clearance [11]; formation of exudates that enhance bacterial growth [7]; and increased bacterial binding to virus infected cells [7]. Most investigators have studied associations of influenza virus and bacteria such as Streptococcus pneumoniae [12], Staphylococcus aureus [13] and Haemophilus influenzae [14,15]. Coincident upper respiratory tract infections with viruses or mycoplasma have been reported to be risk factors for epidemic disease due to group A Neisseria meningitidis [16]; and there is recent epidemiological evidence for an increase in meningococcal disease following epidemics of influenza A [17].

Human respiratory syncytial virus (RSV) is a ubiquitous respiratory tract pathogen which infects 50% of children in their first year of life and virtually all by the age of two [18]. Much of the invasive disease due to meningococci and H. influenzae type b (Hib) occurs in this age group [19].

Although animal models have been used to study disease caused by meningococci and Hib, we chose to use a human epithelial cell line which expresses surface antigens, H and Lewis^b postulated to be involved in bacterial binding [20]. HEp-2, a non-ciliated human epithelial cell line, can be infected with RSV, and uninfected cells have been used for bacterial attachment studies [21,22]. In the present study we tested the hypothesis that HEp-2 cells infected with RSV might bind meningococci or Hib to a greater extent than uninfected cells.

MATERIALS AND METHODS

Cells

HEp-2 cells (Flow Laboratories) were used between passages 400 to 420 for the binding assays. Cell growth medium (GM) consisted of Eagle's minimal essential medium (Gibco) supplemented with 10% foetal calf serum (FCS) (Gibco), $NaHCO_3$ (0.85 g/l), L-glutamine (2mM), streptomycin (200 μg/ml) and penicillin (100 IU/ml).

Virus

RSV (Edinburgh strain) [23] was used at the 5th to 7th passages to infect the HEp-2 cells. Stock aliquots of supernatant fluid from infected cell lysates were stored at -70°C and contained from 1×10^6 - 5×10^6 plaque forming units (pfu)/ml.

Bacteria

Three strains of meningococci were used: C:2b:P1.2; NG:-:P1.9; Y:14:P1.2. Pilate and non-pilate isolates of Y:14:P1.2 were tested. Hib isolates from Icelandic children were kindly provided by Dr K. Jónsdottir, University of Iceland [Table 1]. Serogroup, serotype and subtype of the meningococcal isolates were determined by Dr. R.J. Fallon, Meningococcus Reference Laboratory (Scotland). Cultures were prepared by reconstituting the lyophilized bacteria in distilled

water; meningococci were grown on modified New York City (MNYC) medium [24] and Hib on boiled blood agar. For some experiments meningococci were grown on boiled blood agar or GC agar (Difco, UK) containing 10% lysed horse blood, 2.5% yeast dialysate and 10% glucose to examine the effect of growth medium on attachment. Bacteria were grown overnight at 37°C in 5-10% CO_2 in air. Dulbecco's phosphate buffered saline solution A (DPBSA) was used for washing the bacteria and the cells.

The bacteria were harvested, washed three times in DPBSA by centrifugation at 2500 g for 15 min and resuspended in MM without antibiotics by vigorous pipetting to disperse clumps. The bacterial concentration was determined by measuring optical density (OD) at 540 nm. The linear relationship between OD reading and total count was determined for each strain.

Binding of meningococci to HEp-2 cells in suspension

Overnight monolayer cultures of HEp-2 cells in culture flasks (25 cm^2) were infected with RSV at different multiplicities of infection (MOI) ranging from 0.001 - 1.0 pfu/cell. Virus (1 ml in MM) was adsorbed for 1 hour, replaced with 10 ml MM and incubated overnight at 37°C. Cultures were rinsed twice with DPBSA, then ethylenediaminetetra-acetic acid (EDTA) (0.05%) was applied, 1 ml per flask at 37°C for 5-10 min to produce a cell suspension. MM (5 ml) was then added to the cells to counteract EDTA activity. After centrifugation at 460 g for 7 min, the cells were resuspended in MM without antibiotics, counted and adjusted to 1 x 10^6 cells/ml. The cell suspensions were gently rotated at 37°C whilst the bacteria were prepared. The time from end of viral adsorption to addition of bacteria was 24 h ($\pm$ 1 h).

The bacterial suspensions were labelled with fluorescein isothiocyanate (FITC) (Sigma) freshly prepared as a 0.4% solution in NaH_2CO_3 (0.05M) and NaCl (0.1M) (pH 9.2). The washed bacterial pellet obtained from two culture plates was suspended in 2 ml of the FITC solution by gentle shaking at 37°C for 20 min [25]. FITC-labelled bacteria were washed three times in DPBSA and resuspended in MM without antibiotics. The pilate isolate was incubated at 37°C for 2 h to allow pili to regenerate. The concentration of the suspension was determined by OD at 540 nm and adjusted to provide a range of ratios of bacteria per cell. The bacterial suspensions (200 μl) were incubated with equal volumes of HEp-2 cells at 37°C at 60 rpm in an orbital incubator (Gallenkamp). At the end of each incubation period, the cells were washed three times by centrifugation at 460 g for 7 min. The cells were resuspended in 200 μl of DPBS and fixed with 100 μl of 10% buffered paraformaldehyde (Sigma). The samples were kept in the dark at 4°C until analysed within 3 days of the assay.

Analysis of binding by flow cytometry

Each sample was analysed by flow cytometry with an EPICS-C (Coulter Electronics, Luton, UK) equipped with a 5 watt laser with a power output of 200 mw at 488 nm. The cells were selected from a display of forward angle light scatter (size) versus 90° light scatter (granularity) by means of a bitmap. The bitmap included the main population of the cells excluding unbound bacteria, debris and clumps from further analysis. The percentage of cells with fluorescence greater than background level was recorded on a one parameter histogram (H-%) measuring fluorescence on a logarithmic scale. The mean fluorescence channel values for the positive cells were obtained from a one parameter histogram measuring fluorescence on a linear

scale. After subjecting the H-% histograms to Immunoanalysis (Coulter), a computer programme that subtracts the values of the control population from the test population at each channel of the two H-% histograms, the binding index (BI) of each sample was calculated by multiplying the percentage of fluorescent cells by the mean fluorescence.

RSV infected cells were reacted with mouse monoclonal antibody to RSV-F protein (Kindly supplied by Prof. P.J. Watt, Southampton University) for 30 min at 37°C. After washing, 20 μl of FITC-labelled antimouse IgG antibody (Fab fragment, adsorbed with human serum protein) (Sigma) was added and incubated for 30 min. The cells were washed and fixed in paraformaldehyde and the proportion of cells binding the monoclonal anti-F antibody was determined by flow cytometry.

Binding of H. influenzae to HEp-2 cells

The same method was used to investigate the binding of H. influenzae to HEp-2 cells infected with RSV at MOI 1.0. The bacteria (Table 1) were grown on boiled-blood agar medium for 48 h. FITC-labelled bacteria were incubated with cells in ratios of 50, 200 and 800 bacteria per cell for 2 h, the incubation period recommended by St. Geme and Falkow [26]. The cells were analysed by flow cytometry as described above.

Statistical methods

The results were analysed with the statistical package 'Minitab' using multiple regression on the logarithms of the BIs of the samples. For experiments with meningococci, dummy variables were included to adjust for daily variations and different ratios of viruses and bacteria/cell. The coefficients from this analysis were used to estimate binding relative to that found with 10 bacteria/uninfected cell by taking antilogarithms. Examination of the distribution of residuals from the fitted models suggested that the logarithmic transformation had given an acceptable fit to a normal distribution. A sign test was used to test for consistency of excess binding of H. influenzae to RSV infected cells in repeated experiments.

RESULTS

Detection of RSV infected cells

The percentage of RSV infected cells at 24 h post inoculation for each MOI was determined. This ranged from <10% (MOI = .001) to >80% (MOI = 1) at the time of the attachment assay. No cytopathic effect was noted at this stage.

Attachment of meningococci to cells in suspension assessed by flow cytometry

The effect of RSV infection on binding of meningococci to HEp-2 cells is shown in Figures 1 & 2. The left hand peak in the figures represents fluorescence recorded with uninfected cells following incubation with 320 bacteria/cell. The right hand peak represents fluorescence observed with cells infected with MOI of 0.1 (fig 1) and 1.0 (fig 2) following incubation with 320 bacteria/cell.

Table 2 summarises the results obtained by flow cytometry assays to assess the effect of viral infection at various MOI on binding of bacteria. The results for 11 assays were combined using analysis of

variance and Table 2 shows the derived estimate of the ratios of the binding index obtained with 10 bacteria per uninfected cell taken as 1. The results indicate that prior infection of the cells with RSV at MOI of 0.1 and 1.0 enhanced the binding of N. meningitidis at ratios >80 bacteria/cell as illustrated in figure 3 ($F(2.51) = 37.37$ $P < 0.001$).

At ratios of 10-20 bacteria/cell, the presence of virus infection at any level did not change the binding level significantly. The cells infected with an MOI of 0.001 demonstrated decreased bacterial binding compared with uninfected cells; ($F(1.45) = 5.48$, $P < 0.05$) (figure 4).

The figures and Table 2 were obtained with one strain (C:2b:P1.2) of meningococcus; however, similar results were found with other strains which expressed different serogroup, serotype and subtype surface antigens. Growth on boiled blood agar or GC agar did not alter the pattern of binding obtained when the bacteria were grown on NYC medium. Presence of pili demonstrated by an haenagglutination assay had no effect on the binding pattern.

Attachment of H. influenzae to cells in suspension

Cells infected with RSV at MOI 1.0 bound significantly more Hib than uninfected cells. Although the effect varied widely between experiments, the mean of log differences was positive in all 10 experiments with strain H14 ($P < 0.01$, sign test). RSV infection of the cells enhanced bacterial binding at all the ratios of bacteria to cell tested (Table 3). In 7 additional assays, 4 strains of Haemophilus with different antigenic and biotype characteristics showed a similar increase in binding to RSV-infected cells compared with uninfected cells at each bacterial:cell ratio tested.

DISCUSSION

Increased susceptibility to secondary bacterial disease following viral infection has been recognized for many years [27]. Among the factors suggested to contribute to susceptibility to secondary infections, the enhancement of bacterial binding to virus-infected cells might be an important initial step.

In initial experiments, binding of meningococci to HEp-2 cells and RSV-infected cells was assessed by light microscopy. The method was not used for quantitative studies because of wide variation in the counts obtained (data not shown) and the time needed for the assay. The experiments were, however, helpful to demonstrate binding of the bacteria to the cells and to determine optimal incubation periods before cytotoxic effects occurred. These efects might be the direct effect of the bound bacteria [21] or of the release of lipopolysacchride from the bacteria [28]. Although increased binding of bacteria to RSV infected cells was observed with this technique, results were not reliably reproducible. In our studies, measuring fluorescein-labelled bacteria on the surface of cells by flow cytometry is a more sensitive and precise method than microscopy. More cells can be counted in a short period of time and there is no subjectivity in the values recorded.

The finding that in the cell samples with <10% infected cells (MOI 0.001) meningococcal binding is significantly decreased compared with

uninfected cells was not expected (figure 4). The RSV-infected cells might be secreting a soluble factor such as interferon which results in reduced bacterial binding to neighbouring uninfected cells in the sample. Compared with controls, total bacterial binding was not significantly altered for the samples containing 20-30% infected cells (MOI 0.01). This might reflect the mixed population of infected cells and uninfected cells.

Enhanced binding of meningococci and haemophilus to cells infected with RSV at MOI $\geq$ 0.1 pfu/ml 24 hours previously suggests four possibilities. RSV infection might enhance the expression of existing receptor(s) for the bacteria. It might induce expression of a new cell receptor. The filaments observed on the surface of RSV-infected cells might trap the bacteria [29]. The F or G viral glycoproteins expressed on the infected cells might act as additional receptors for the bacteria. Because Hep-2 cells infected with RSV express viral glycoproteins F and G on their surface at the time used in these experiments, the last hypothesis is currently under investigation.

The results of this study suggest virus infection might be a predisposing factor for bacterial colonization of epithelial cells. Epidemiological investigations are underway to assess the influence of RSV infection on carriage of meningococci or Hib.

ACKNOWLEDGEMENTS

MWR is a recipient of a scholarship from the Ministry of Education and Technology of the government of Pakistan and an Overseas Research Scholarship (ORS) award. This work is supported by the National Meningitis Trust and TENOVUS-scotland.

REFERENCES

1. Krasinski K, Nelson JD, Butler S, Luby JP, Kusmiesz H. Possible association of mycoplasma and viral respiratory infections with bacterial meningitis. Am J Epidem 1987; 125(3): 499-508.
2. Ramírez-Ronda CH, Fuxench-López Z, Névarez M. Increased pharyngeal bacterial colonization during viral illness. Arch Int Med 1981; 141: 1599-1603.
3. Sanford BA, Davison VE and Ramsay MA. Staphylococcus aureus adherence to influenza A virus infected and control cell cultures: Evidence for multiple adhesions. Proc Soc Exp Biol Med 1986; 181: 104-111.
4. Young LS, La Force FM, Head JJ, Felley JC and Bennet JV. A simultaneous outbreak of meningococcal and influenza infection. New Eng J Med 1972; 287: 5-9.
5. Gwaltney JM, Sande MA, Austrian R, Hendley JO. Spread of Streptococcus pneumoniae in families. II. Relation of transfer of S. pneumoniae to incidence of colds and serum antibody. J Inf Dis 1975; 132(1): 62-68.
6. Hietella J, Uhari M, Tuokko H, Leinonen M. Mixed bacterial and viral infections are common in children. Pediatric Infect Dis J 1989; 8(10): 683-6.
7. Babiuk LA, Lawman MJP, Ohmann HB. Viral-bacterial synergistic interaction in respiratory disease. Adv Vir Res 1988; 35: 219-249.
8. Jakab GJ. Immune impairment of alveolar macrophage phagocytosis during influenza virus pneumonia. Am Rev Resp Dis 1982; 126: 778-782.
9. Jakab GJ. Mechanisms of virus-induced bacterial superinfections of the lung. Clin Chest Med 1981; 2: 59-66.
10. Martin RR, Couch RB, Greenberg SB, Cate TR, Warr GA. Effects of infection with influenza virus on the function of polymorphonuclear leucocytes. J Inf Dis 1981; 144(3): 279.
11. Camner P, Jarstrand C, Philipson K. Tracheobronchial clearance in patients with influenza. Am Rev Res Dis 1973; 108: 131-5.
12. Plotkowski M, Puchelle E, Beck G, Jacquot J, Hannoun C. Adherence of type 1 Streptococcus pneumoniae to tracheal epithelium of mice infected with influenza A/PR8 virus. Am Rev Resp Dis 1986; 134: 1040-1044.
13. Musher DM, Fainstein V. Adherence of Staphylococcus aureus to pharyngeal cells from normal subjects, smokers, staphylococcal carriers and patients with viral infections. In: Jeljaszewcz J, editor. Staphylococci and staphylococcal infections. New York: Gustav Fischer Verlag, Zbl Bak (Suppl 10), 1981: 1011-1016.
14. Bakaletz LO, Hoepf TM, DeMaria TF, Lim DJ. The effect of antecedent influenza A virus infection on the adherence of Haemophilus influenzae to chinchilla tracheal epithelium. Am J Otolaryngol 1988; 9: 127-134.
15. Fainstein V, Musher DM, Cate TR. Bacterial adherence to pharyngeal cells during viral infection. J Inf Dis 1980; 141(2): 172-6.
16. Moore PS, Heirholzer J, DeWitt W, Goovan K, Djore D, Lippeveld T, et al. Respiratory viruses and mycoplasma as co-factors for epidemic group A meningococcal meningitis. JAMA 1990; 264(10): 1271-5.

17. Cartwright KAV, Jones DM, Smith AJ, Stuart JM, Kaczmarski EB, Palmer SR. Influenza A and meningococcal disease. Lancet 1991; 338: 554-7.
18. Henderson FW, Collier AM, Clyde WA Jr., Denny FW. Respiratory Syncytial Virus infection, reinfection and immunity: a prospective longtudinal study in young children. N Eng J Med; 1979; 300: 530-534.
19. Greenwood BM. Selective primary health care: Strategies for control of disease in the developing world. XIII. Acute bacterial meningitis. Rev Inf Dis 1984; 6(3): 374-389.
20. Blackwell CC. The role of ABO blood groups and secretor status in host defences. FEMS Microbiol Immunol 1989; 47: 341-350.
21. Stephens DS. Gonococcal and meningococcal pathogenesis as defined by human cell, cell culture and organ culture assays. Clin Mcrobiol Rev 1989; suppl 2: s104-s111.
22. Layh-Schmitt G, Schmitt S, Buchanan TM. Interaction of non-pilate Neisseria gonorrhoeae strain 7122 and protein IA with an epithelial cell monolayer. Int J Med Microbiol 1989; 272(2): 158-170.
23. Ogilvie MM, Vathenen AS, Radford M, Codd J and Key S. Maternal antibody and respiratory syncytial virus infection in infancy. J Med Virol 1981; 7: 263-271.
24. Young H. Cultural diagnosis of gonorrhoea with modified New York City (MNYC) medium. Br Med J Vener Dis 1978; 54: 36-40.
25. Rahat A. Studies on the role of blood group antigens in susceptibility to meningococcal diseases (PhD Thesis) 1990. Edinburgh University UK; 83-84.
26. St. Geme JW, Falkow S. Haemophilus influenzae adheres to and enters cultured human epithelial cells. Infect Immun 1990; 58(2); 4036-44.
27. Nichol KP, Cherry JD. Bacterial-viral interrelations in respiratory infections of children. N Eng J Med 1967; 277(13): 667-672.
28. Stephen DS, Hoffman LH, McGee ZA. Interaction of Neisseria meningitidis with human naso-pharyngeal mucosa: attachment and entry into columnar epithelial cells. J Inf Dis 1983; 148(3): 369-376.
29. Parry JE, Shirodria PV and Pringle CR. Pneumoviruses: the cell surface of lytically and persistently infected cells. J Gen Virol 1979; 44: 479-491.

Table 1. Isolates of *N. meningitidis* examined

Serogroup	Serotype	Subtype	Source
C	2b	P1.2	patient
NG	-	P1.9	carrier
Y*	14	P1.2	carrier

* This strain was used in both pilate and non-pilate forms.

Table 2. Isolates of type b *Haemophilus influenzae* examined

Strain no.	Source	Enzyme type	Biotype	OMP type	LPS type
14	Edinburgh	-	-	-	-
20	Reykjavik	4b	I	NT	1
21	Reykjavik	1	I	2	9
25	Reykjavik	12	I	1	1
29	Keflavik	1	-	-	-

NT = non typable

Table 3. Effects of infection of HEp-2 cells with RSV on binding of *N. meningitidis*

No. of bacteria added per cell	Estimated binding indices from multiple regression				
	Amount of virus added (MOI)				
	0	0.001	0.01	0.1	1.0
10*	100*	60	69	125	-
20	79	89	123	102	117
40	194	123	125	239	213
80	316	190	323	331	407
160	575	380	467	776	1288
320	1000	588	1148	1905	3311
640	1318	-	-	2818	5248
1000	2238	-	-	5623	11220

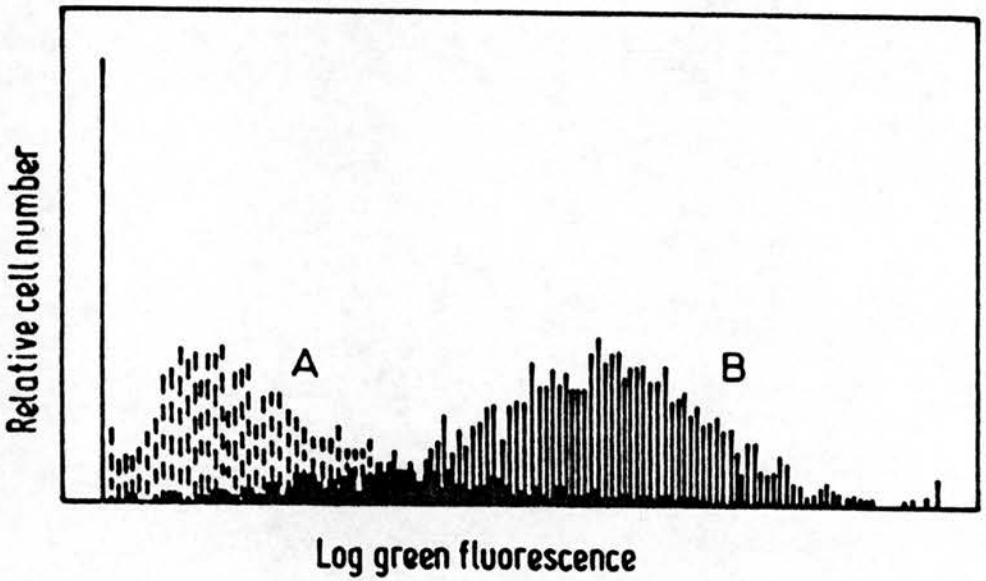
(* mean for MOI = 0 and 10 bacteria per cell taken as 100%)

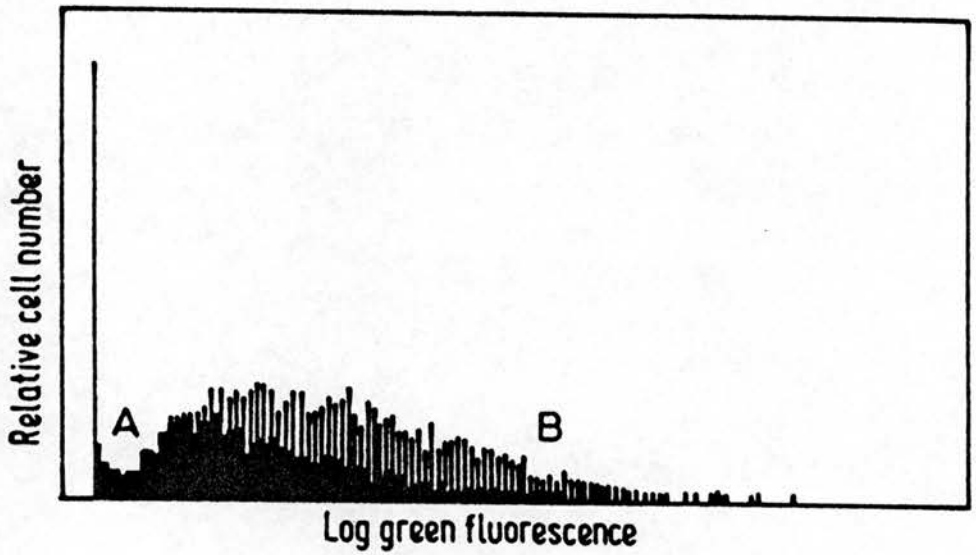
Figure 1. Binding of FITC labelled *N. meningitidis* to HEp-2 cells infected with RSV (MOI 0.1) (B) and uninfected cells (A).

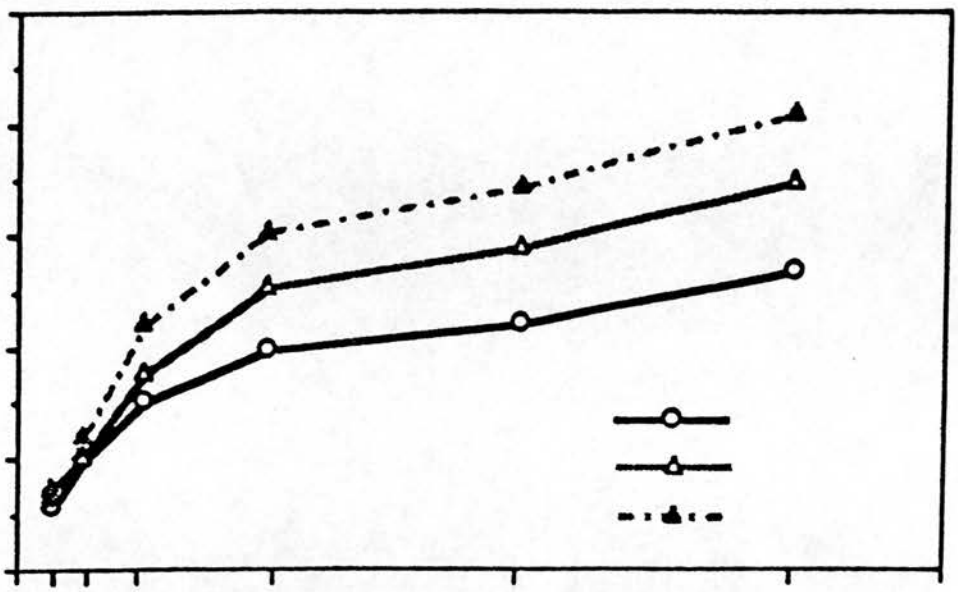
Figure 2. Binding of FITC labelled *N. meningitidis* to HEp-2 cells infected with RSV (MOI 1.0) (B) and uninfected cells (A).

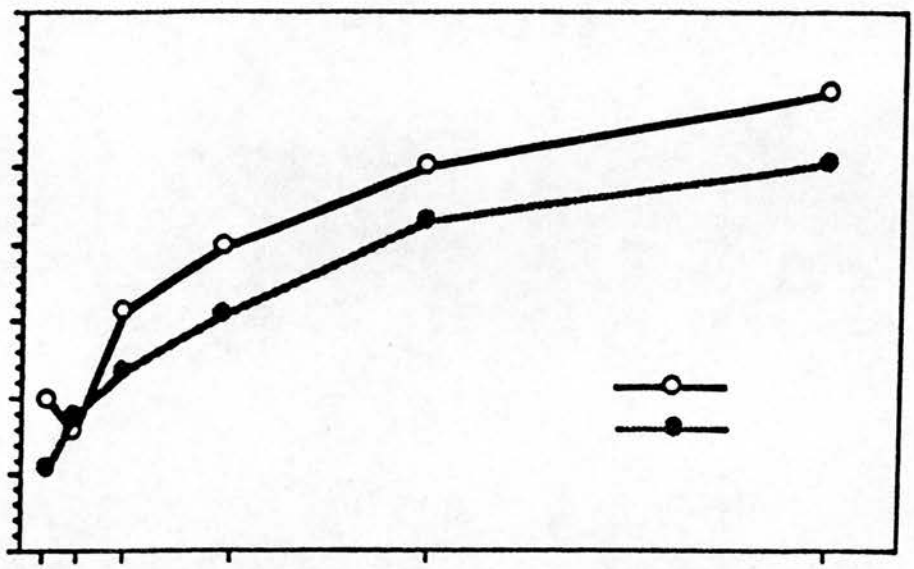
Figure 3. Effect of infection of HEp-2 cells with RSV at MOI of 0.1 and 1.0 on binding of *Neisseria meningitidis* to the cells.

Figure 4. Effect of infection of HEp-2 cells with RSV at MOI of 0.001 on binding of *Neisseria meningitidis* to the cells.


Relative cell number
A
B
Log green fluorescence


Relative cell number
A
B
Log green fluorescence





FACTORS ENHANCING ADHERENCE OF TOXIGENIC STAPHYLOCOCCUS AUREUS TO EPITHELIAL CELLS AND THEIR POSSIBLE ROLE IN SUDDEN INFANT DEATH SYNDROME

A.T. Saadi, C.C. Blackwell, M.W. Raza, V.S. James, J.Stewart, R.A.Elton* and D.M.Weir

Department of Medical Microbiology
University of Edinburgh
Medical School
Teviot Place
Edinburgh EH8 9AG

*Medical Statistics Unit
Department of Public Health Sciences
University of Edinburgh
Medical School
Teviot Place
Edinburgh EH8 9AG

Running head : Toxigenic S. aureus and Lewis^a in SIDS.

ref: ccb/mc/tc/sids2

ABSTRACT

Toxigenic strains of Staphylococcus aureus have been suggested to play a role in Sudden Infant Death Syndrome (SIDS). In this study we examined two factors that might enhance binding of toxigenic staphylococci to epithelial cells of infants in the age range in which cot deaths are prevalent: expression of the Lewis^a antigen and infection with respiratory syncytial virus (RSV). By flow cytometry we demonstrated that binding of three toxigenic strains of S. aureus to cells from non-secretors was significantly greater than to cells of secretors. Pre-treatment of epithelial cells with monoclonal anti-Lewis^a or anti-type 1 precursor significantly reduced bacterial binding ($P < 0.01$); however, attachment of the bacteria correlated only with the amount of Lewis^a antigen detected on the cells ($P < 0.01$). HEp-2 cells infected with RSV bound significantly more bacteria than uninfected cells. These findings are discussed in context of factors previously associated with SIDS (mother's smoking, bottle feeding and the prone sleeping position) and a hypothesis proposed to explain some cases of SIDS.

INTRODUCTION

The suggestion that microorganisms are involved in the aetiology of some cases of sudden infant death syndrome (SIDS) is based on evidence from a variety of epidemiological and pathological studies. SIDS occurs during the period when maternal antibodies have declined and the infant immune system is immature. There is a marked seasonal variation in SIDS; the risk increases in autumn and winter when respiratory infections are more common [1,2]. There is often a history of upper respiratory tract infection in these infants [3]. Mother's smoking was identified as a factor for SIDS in the New Zealand studies [4]. Smoking and passive exposure to cigarette smoke have been associated with increased risk of respiratory tract infections [5] and with carriage of potentially pathogenic microorganisms [6,7]. At post mortem examination, there is often evidence of minor inflammation and infection of the respiratory tract in many SIDS infants [8].

There are conflicting results on the role of viruses [9,10] and toxigenic intestinal bacteria in SIDS [11-15]. Recently, it has been suggested that nasopharyngeal colonization by toxigenic strains of Staphylococcus aureus, particularly those producing the toxic shock syndrome toxin 1 (TSST-1), might be involved in some of these infant deaths [16]. Pyrogenic toxins are produced by many strains of S. aureus, and similar ones are found among some strains of group A beta-haemolytic Streptococcus pyogenes. These are "superantigens" that have significant physiological effects such as induction of fever (> 38°C), possibly by direct action on the hypothalamus or by their induction of tumor necrosis factor (TNF) and interleukin 1 by monocytes [17]. S. aureus was isolated from a higher proportion of SIDS infants (41.3%), compared with a control population (28.3%) [18]. In this study we examined two factors that might enhance density of colonization by toxigenic staphylococci in infants during the period when they are at risk of SIDS: expression of the Lewis^a blood group antigen, and infection with respiratory syncytial virus (RSV).

Non-secretion, the genetically controlled inability of an individual to secrete the glycoprotein form of his/her ABO blood group antigens, is associated with susceptibility to a number of bacterial diseases and with asymptomatic carriage of some potentially pathogenic microorganisms [19], including group A streptococci [20]. The secretor gene also influences expression of the Lewis blood group antigens; non-secretors produce only Lewis^a; secretors produce Lewis^b predominantly but also variable amounts of Lewis^a. The amount of Lewis^a present in secretors depends on the relative activities of the fucosyl transferases coded for by the secretor gene and the Lewis gene. If the secretor fucosyl transferase acts first on the precursor chain, the Lewis enzyme can add fucose to the subterminal sugar of the chain to produce Lewis^b. If the Lewis fucosyl transferase acts on the precursor first, the Lewis^a molecule cannot act as a substrate for the secretor enzyme.

One of the hypotheses proposed to explain the increased proportion of non-secretors among carriers of some potentially pathogenic bacteria and yeasts is that the Lewis^a antigen is one of the host cell receptors for some microorganisms [19,21,22]. In this context, the reported high proportion of infants expressing Lewis antigens during the first year of life was of particular interest [23]. Among infants, the enzyme coded for by the secretor gene appears to be less efficient than that coded for by the Lewis gene. Although the majority of infants are secretors (75-80%), during the first year of life they will express easily detectable amounts

of Lewis^a. The peak in the proportion of infants expressing Lewis^a is 2-4 months, similar to the highest incidence of cot deaths.

It has been reported that both natural and experimental viral infections enhance colonization by S. aureus [24]. RSV infected cells bind greater numbers of Neisseria meningitidis and type b Haemophilus influenzae compared with uninfected cells [25]. As RSV infects almost half of all infants by the age of 12 months and is most prevalent in the winter months [26], it is an obvious candidate for investigation of the role viruses might play in enhanced colonization by toxigenic bacteria.

These observations prompted the following questions:

1. Is Lewis^a detected in SIDS infants?
2. Do strains of toxigenic S. aureus bind in greater numbers to non-secretor cells expressing larger amounts of Lewis^a ?
3. As some viral infections increase carriage of staphylococci, do tissue culture cells infected with RSV bind greater numbers of staphylococci than uninfected cells?

The results of these investigations are discussed in the context of epidemiological data on the role of microorganisms in SIDS.

SUBJECTS AND METHODS

Respiratory tract secretions (89) obtained from SIDS infants during autopsy were provided by Dr. J.N. Inglis and Dr. P. Molyneaux (Regional Virus Laboratory, City Hospital, Edinburgh) and by Dr. A. Gibson (Royal Hospital for Sick Children, Glasgow). These were examined for presence of H [27] and Lewis antigens [28] by enzyme-linked immunoassays (ELISA) described previously.

S. aureus strains NCTC 10652, NCTC 10654, NCTC 10655, NCTC 10656, NCTC 10657, NCTC 11965, and NCTC 8532 were obtained from Dr. A. Wieneke Central Public Health Laboratory, Colindale. Strains 40654 and 41206 were kindly provided by Dr. J. Medcraft, Public Health Laboratory Service, Department of Microbiology, Reading, Berkshire. The strains and the toxins they produced are listed in Table 1. The bacteria were grown on nutrient agar or for some experiments on blood agar to examine the effect of medium on binding to epithelial cells

Buccal epithelial cells (BEC) were obtained from pairs of healthy secretor and non-secretor donors matched as closely as possible for ABO blood group, age and sex. ABO groups of the donors were determined by slide agglutination of erythrocytes with monoclonal anti-A and anti-B antibodies (Scottish National Blood Transfusion Service). Secretor status was determined from saliva by haemagglutination inhibition assays [29], initially confirmed by tube agglutination with monoclonal anti-Lewis^a or anti-Lewis^b antibodies (Scottish National Blood Transfusion Service) and later by ELISA's for H and Lewis antigens.

BEC were collected by rubbing the inside of the cheeks with cotton swabs. To remove the cells, the swabs were agitated in 10 ml phosphate buffered saline (PBS) (pH 7.2). They were washed twice in PBS in a Sorvall RT 6000 centrifuge (300g for 10 minutes) and the concentration adjusted to 2×10^5 ml^{-1} after determination of the number of cells microscopically in an improved Neubauer haemocytometer.

Bacteria were labelled with fluorescein isothiocynate (FITC) by a modification of the method of Wright and Jong [30]. A heavy suspension of bacteria in PBS was prepared and washed twice by centrifugation at 1000 g for 20 minutes. The bacterial pellet was resuspended in 4 ml of a freshly prepared 0.04% solution of FITC in sodium carbonate (0.05M) and sodium chloride (0.1M) (pH 9.2). The mixture was incubated at 37°C for 20 min and washed twice with PBS. The pellet was resuspended in PBS and filtered through a Millipore membrane filter (5um pore size) to remove clumps of bacteria.

The bacterial concentration was determined by measuring the optical density of the suspension at 540 nm. The linear relationship between optical density and total count was determined for each strain.

Binding of bacteria to BEC of secretors and non-secretors

BEC (200 ul) were mixed with 200 ul of the FITC-labelled bacteria at the following ratios of bacteria per cell: 80:1, 160:1 and 320:1. The mixtures were incubated at 37°C for 30 min with gentle shaking in an orbital incubator (Gallenkamp). The cells were washed twice with PBS by centrifugation at 300g for 10 minutes to remove unattached bacteria. The

samples were resuspended in 300 ul 1% buffered paraformaldehyde (Sigma) and stored in the dark at 4°C until analysed.

Analysis of binding assays

Each sample was analyzed by flow cytometry with an EPICS-C (Coulter Electronics, Luton, UK) equipped with a 5 watt laser with a power output of 200 mw at 488 nm. Cells were selected from a display of forward angle light scatter (size) versus 90° light scatter (granularity) by means of a bitmap. The bitmap included the main population of the cells and excluded debris and clumps from further analysis. The percentage of cells with fluorescence greater than background level was recorded on a one parameter histogram which measured fluorescence on a lograthmic scale. The mean fluorescence channel values for positive cells were obtained from a one parameter histogram measuring fluorescence on a linear scale. The results were analyzed by Immunoanalysis (Coulter), a computer programme that subtracts the values of the control population from the test population at each channel of the two histograms. The binding index (BI) of each sample was calculated by multiplying the percentage of fluorescent cells by the mean fluorescence channel value.

Inhibition of bacterial binding

BEC obtained from secretors or non-secretors were treated with monoclonal anti-Lewis^a (LM112/161.8, kindly supplied by Dr. R.H. Fraser, Law Hospital, Carluke) or monoclonal antibody to the type 1 precursor chain (anti-precursor type 1) (clone no. 619/102, Russel Fine Chemicals, Chester) for 60 min at 37°C. After washing twice by centrifugation at 300g for 10 min, the attachment assay was performed and analysed as described above.

Binding of bacteria to HEp-2 cells

Methods for assessment of binding of Staph. aureus strains NCTC 8532 and NCTC 10655 to uninfected and RSV-infected HEp-2 tissue culture cells and analysis of results were as described for H.influenzae [25].

Detection of Lewis^a and type 1 precursor antigens on BEC of secretor and non-secretor donors.

Buccal epithelial cells (200 ul, 2 x $10^5 ml^{-1}$) from seven secretors, seven non-secretors and three secretor donors whose erythrocytes were not agglutinated by either anti-Lewis^a or anti-Lewis^b monoclonal antibodies (Scottish National Blood Transfusion Service) (Lewis-negative individuals) were incubated with 200 ul of monoclonal antibodies to Lewis^a (1/5) or precursor type 1 (1/10) for 60 min at 37°C. The cells were washed twice with PBS at 300 g for 10 min and incubated with 200 ul rabbit anti-mouse IgM conjugated with FITC (1/200) (Sigma). The FITC-labelled antibody was also added to 200 ul of cells which had not been treated with the first antibody as a control. After 30 min incubation in an orbital shaker at 37°C, the cells were washed twice with PBS. They were resuspended in 300 ul 1% buffered paraformaldehyde and stored in the dark at 4°C until analysed. The cells were analysed on an EPICS-C flow cytometer as described above and the binding indices calculated as before.

Statistical methods

All analyses were carried out on the logarithms of the binding indices which conformed more closely to a normal distribution than the raw values. Differences between groups were tested by paired or unpaired t-tests as appropriate, and confidence limits for the mean values in one group were expressed as a percentage of those in the other by taking antilogarithms. Analysis of covariance was used to test whether binding levels were associated with the amount of anti-Lewis[a] in secretors and non-secretors tested on different days, while the association in a similar experiment with anti-precursor on a single day was tested by Pearson correlation. Three-factor analysis of variance was used to test whether RSV infection was related to binding of HEP2 cells at two different concentrations of bacteria per cell.

RESULTS

Detection of Lewis^a antigen in body fluids of SIDS infants

Lewis^a antigen was detected in 63/89 (71%) of the specimens from SIDS infants.

Binding of *S. aureus* to epithelial cells of secretors and non-secretors

The results of the binding assays are presented in Table 1 as the 95% confidence limits for binding of the bacteria to non-secretor cells expressed as a percentage of binding to cells from matched secretors. Confidence intervals for which both values are above 100 indicate significant evidence that cells from non-secretors bound more staphylococci than cells from secretors. There was no significant difference in binding to cells of non-secretors compared with binding to cells of secretors observed for the non-toxigenic strain NCTC 8532 or five of the toxin producing strains: 41206, 40654, NCTC 10654, NCTC 10656 and NCTC 10652. Three of the toxigenic isolates NCTC 10655, NCTC 11965 and NCTC 10657, showed higher binding to cells from non-secretors compared with binding to cells from secretors (Table 1). There was significantly higher binding of NCTC 10655 and NCTC 10657 to non-secretor cells at all ratios of bacteria:cells tested, while for strain NCTC 11965 there was significantly higher binding to cells of non-secretors only with the lowest ratio of bacteria.

Binding of anti-Lewis^a or anti-precursor antibody to epithelial cells of secretors and non-secretors

The epithelial cells from the 3 individuals whose erythrocytes were not agglutinated by either anti-Lewis^a or anti-Lewis^b (Lewis-negative) bound consistantly low levels of anti-Lewis^a. Cells from non-secretors bound high levels of the antibody. While the mean binding index for secretors (36,707) was approximately half that for non-secretors (79,148), there was considerable variation in the amount of antibody bound by individual secretors (Figure 1). There was no consistent pattern of binding of the anti-precursor monoclonal to cells of secretors, non-secretors or Lewis-negative individuals

Inhibition of bacterial binding

Binding of S. aureus NCTC 10655 to BEC obtained from seven secretors or seven non-secretors treated with anti-Lewis^a antibody was significantly lower compared with binding of the bacteria to untreated cells from the same donors ($t=-4.46$, $df=13$, $P<0.001$ 95% CI 55% - 81%). A similar pattern was observed for binding of NCTC 10655 to cells of seven non-secretors and ten secretors treated with monoclonal anti-precursor type 1 compared with untreated cells ($t = 8.19$, $df = 16$, $P < 0.001$; 95% CI 58% - 72%).

Binding of S. aureus NCTC 8532 to the cells from the same donors treated with anti-Lewis^a antibody was significantly lower compared with binding of the bacteria to untreated cells ($t = -4.97$, $df = 11$, $P < 0.001$ 95% CI 43% - 72%). Inhibition of NCTC 8532 with anti-precursor was not examined because of the limited amount of antibody.

Binding of bacteria with reference to detection of Lewis^a antigen or precursor antigen

Binding of S. aureus NCTC 10655 was correlated with the amount of monoclonal anti-Lewis^a antibody detected on the BEC of secretors and non-secretors ($df = 12$, $t = 5.03$, $P < 0.001$). A similar pattern was found with NCTC 8532 ($t = 4.24$, $df = 12$, $P < 0.001$). There was no significant correlation between the amount of anti-precursor detected on epithelial cells and binding of NCTC 10655 or NCTC 8532.

Binding of S. aureus to RSV infected cells

Strains NCTC 8532 and NCTC 10655 were assessed in seven experiments to compare binding of bacteria to HEp-2 cells and RSV-infected HEp-2 cells. For both isolates the binding indices were greater for the virus infected cells: NCTC 8532 ($P < 0.001$, 95% CI 117% - 155%), NCTC 10655 ($p < 0.05$, 95% CI 109% - 155%).

DISCUSSION

The results provided information for the three questions to be investigated in this study. Lewis^a antigen was detected in secretions of 71% of SIDS infants examined. There was increased binding of some toxigenic strains of S. aureus to epithelial cells from non-secretor donors; and RSV-infected cells bound more staphylococci than uninfected cells.

The lack of relevant control populations is a major criticism of epidemiological studies of cot deaths. There is, however, evidence from several surveys that S. aureus is isolated from the nasopharynx of approximately 35-40% of SIDS infants [16,18,31] compared with 28% from healthy infants [18]. TSST-1 has been demonstrated in the renal tubular cells of some SIDS infants but not in a comparison group of infants who were not cot death victims [32]. The pyrogenic toxins of S. aureus and group A streptococci are powerful "superantigens" that can induce release of cytokines that might trigger a cascade of events leading to shock or damage to the respiratory or cardiac systems [17].

Because density of colonization might be an important consideration in the hypothesis that these toxins play a role in some cot deaths, factors suggested to enhance colonization were assessed in the study reported here. Non-secretors of ABO blood group antigens are over-represented among carriers of group A streptococci [20]. We tested the hypothesis that epithelial cells from non-secretor donors might bind greater numbers of toxigenic staphylococci than cells from secretors. Three of the eight toxigenic strains, including one producing TSST-1, did show significantly higher binding to non-secretor cells.

Attachment of strain 10655 which bound in greater numbers to non-secretor cells was significantly inhibited by pretreatment of the host cells with either monoclonal anti-Lewis^a antibody or monoclonal anti-precursor type 1 antibody. Anti-precursor type 1 antibody was examined because it has been suggested that bacteria might bind to the precursor portion of the ABO or Lewis blood group antigens; and glycosylation of the precursor to H or Lewis antigens decreased the accessibility of the binding site [33]. There was a significant correlation between binding of 10655 and binding of anti-Lewis^a antibody to the host cell, but there was no correlation between binding of this strain with the amount of anti-precursor antibody detected on the cells. As the anti-precursor monoclonal was prepared by immunization with Lewis ^{a} antigen, the inhibition of bacterial binding observed following pretreatment of cells with anti-precursor might be due to cross-reactivity with Lewis^a although none was indicated by the manufacturer.

Although NCTC 8532 did not bind in greater numbers to non-secretor cells compared with secretor cells, its binding was inhibited by treatment of the epithelial cells with anti-Lewis^a and there was a significant correlation between its binding to the donor cells and the amount of anti-Lewis^a antibody bound by the cells. From figure 1, it is apparent that some secretors bind as much anti-Lewis^a as most non-secretors. For the experiments comparing the binding of staphylococci to cells from secretors and non-secretors, the donors were matched only according to sex, age and ABO blood group; and the same pairs of secretor/non-secretor donors were not available for each experiment. At that time there was no information on the amount of anti-Lewis^a bound by the cells of individual donors.

Early experimental work suggested that there are multiple receptors for staphylococci on human epithelial cells [34]. If Lewis[a] is one of the receptors for some strains of staphylococci or group A streptococci capable of producing pyrogenic toxins, the expression of this antigen among young infants might enhance their colonization by these bacteria. Viral infections have been shown to enhance carriage of staphylococci [24]; and disease due to TSST-1 has been reported to follow influenza or "flu-like" illnesses [35]. Binding of both meningococci and type b H. influenzae to tissue culture cells derived from human epithelium (HEp-2) was substantially increased if the cells were infected with RSV [25]; and, in this study, both the toxigenic and non-toxigenic strain tested bound in greater numbers to the RSV infected cells. If there are similar interactions in vivo, infection with RSV common during the first year of life might also enhance colonization by these bacteria.

These studies suggest two factors that might enhance staphylococcal colonization of young infants; however, all infants who become colonized do not become SIDS victims. The following hypothesis is an attempt to correlate our laboratory findings with factors identified in epidemiological studies of cot deaths in New Zealand: mother's smoking, prone sleeping position and bottle feeding [4].

Mother's smoking which was associated with carriage of meningococci among children [36] might increase the risk of initial exposure to potentially pathogenic bacteria in two ways. First, epithelial cells from smokers have been shown to bind greater numbers of staphylococci than cells from non-smokers [37]. Smoking also enhances susceptibility to respiratory viral infections; and epithelial cells from indiviudals with natural or experimental viral infections bound more staphylococci compared with those from individuals who were not infected with a virus [37].

In addition to the two factors examined in the present study, others that might enhance density of colonization of infants by staphylococci include passive exposure to cigarette smoke which decreases mucocilary clearance. Infants in the age range in which the peak of SIDS occurs have little or no mucosal or systemic immunity to staphylococci or to the pyrogenic toxins. The effect of breast feeding on carriage of staphylococci or susceptibility to these toxins is unknown; however, in studies on the possible role of toxigenic clostridia in cot deaths, Clostridium difficile was isolated from significantly fewer breast fed infants compared with formula fed infants, and Cl. difficile toxin was detected only in the faeces of formula fed infants [38].

The pyrogenic toxins are produced between 37-40° C; and they are produced in greater quantities at the higher temperatures [17]. Three factors associated with SIDS might increase the infant's temperature and thereby enhance toxin production: respiratory infection; overwrapping with clothing or bedding; and the prone sleeping position. In the prone sleeping position, infants lose less heat than in the supine position. [39,40,41]. The synergistic effect between increased temperature and increased toxin production might account for the high temperatures recorded for some of these infants at autopsy. Among 24 infants who died suddenly and whose rectal temperatures were measured immediately before refrigeration, 10 had temperatures > 38°C and 5 were > 40°C [42]. In addition to the heat shock hypothesis of cot death, the increased release of IL-1 induced by the toxins might contribute to prolonged sleep apnoea as suggested by Guntheroth [43].

Studies are underway to assess levels of Lewis^a in saliva of infants, exposure to cigarette smoke and presence of RSV on carriage of toxigenic staphylococci and streptococci. Results of these studies should provide evidence to refute or confirm the scheme proposed.

ACKNOWLEDGEMENTS

This work was supported by a grant from the Scottish Cot Death Trust. MWR is a recipient of a postgraduate studentship from the government of Pakistan and an Overseas Research Scholarship (ORS) award.

REFERENCES

1. Froggat P, Lynas MA, Mackenzie G. Epidemiology of sudden unexpected death in infants in N. Ireland. Br J Paediatr Soc Med 1971; 25: 119-134.
2. Carpenter RG, Gardner A. Variations in unexpected infant death rates relating to age, sex and season. Studies on medical and population subjects. No. 45, 1982; 23-31; HM Stationery Office, London.
3. Valdes-Dapena MA. A review of the world literature 1954-1966. Paediatrics 1967; 39: 123-138.
4. Mitchel EA. Review Article: cot-death : should the prone sleeping position be discouraged? J Paediatr Child Health 1991; 27: 319-321.
5. Bonham GS, Wilson PW. Childrens health in families with cigarette smokers. Am J Public health 1981; 71: 290-293.
6. Stuart JM, Cartwright KAV, Robinson PM, Noah ND. Effect of smoking on meningococcal carriage. Lancet 1989; ii: 723-725.
7. Blackwell CC, Weir DM, James VS et al. Secretor status, smoking and carriage of Neisseria meningitidis. Epidemiol Infect 1990; 104: 203-209.
8. Berry PJ, Keeling JW. The investigation of sudden infant death in infancy. In : Recent advances in histopathology. (Eds) PP Anthony, RNM MacSween, London, Churchill Livingstone; 1989.
9. Scott DJ, Gardner PS, McQuillan J, Stanton AN, Downham MAPS. Respiratory viruses and cot death. BMJ 1978; ii: 12-13.
10. Williams AL, Uren EC, Bretherton L. RSV and SIDS. Brit Med J 1984; 288: 1491-1493.
11. Arnon SS, Midura TF, Damus K, Wood RM. Intestinal infection and toxin produced by Cl. botulinum as one cause of SIDS. Lancet 1978; 1273-1277.
12. Arnon SS, Damus K, Chin J. Infant botulism: epidemiology and relation to SIDS. Epidemiol Rev 1981; 3: 45-66.
13. Arnon SS. Breast feeding and toxigenic intestinal infections: missing links in crib death? Rev Infect Dis 1984; 6 Suppl 1: S193-201.
14. Sonnabend OAR, Sonnabend WFF, Krech U, Molz G, Sigrist T. Continuous microbiological and pathological study of 70 sudden and unexpected infant deaths: toxigenic intestinal Clostridium botulinum infection in 9 cases of SIDS. Lancet 1985; 2: 237-241.
15. Turner HD, Brett EM, Gilbert RJ, Ghosh AC, Liebeschnetz HY. Infant botulism in England. Lancet 1978; i: 1277-1278.
16. Morris JA. Hypothesis: common bacterial toxins are a possible cause of SIDS. Medical Hypothesis 1987; 22: 211-222.
17. Bohach GA, Fast DJ, Nelson RD, Schlievert PM. Staphylococcal and streptococcal pyrogenic toxins involved in toxic shock syndrome and related illnesses. Crit Rev Microbiol 1990; 17: 251-272.
18. Telford DR, Morris JA, Hughes P, Conway AR, Lee S, Barton AJ, Drucker DB. The nasopharyngeal bacterial flora in the SIDS. J Infect 1989; 18: 125-130.
19. Blackwell CC. The role of ABO blood groups and secretor status in host defences. FEMS Microbiol Immunol 1989; 47: 341-350.
20. Haverkorn MJ, Goslings WRO. Streptococci, ABO blood groups and secretor status. Am J Hum Genet 1969; 21: 360-375.
21. May SJ, Blackwell CC, Weir DM. Lewis^a blood group antigen of non-secretors: a receptor for Candida blastospores. FEMS Microbiol Immunol 1989; 47: 407-410.

22. Rahat A. Studies on the Role of Blood Group Antigens in Susceptibility to Meningococcal Infection. [PhD Thesis]. University of Edinburgh 1990.
23. Issit PD. The Lewis system in applied blood serology. 3rd edition. Miama: Montogomery Scientific Publications 1985; pp 169-191.
24. Ramfrez-Ronda CH, Fuxench-Lopez A, Nevarez M. Increased bacterial colonisation during viral illness. Arch Intern med 1981; 141: 1599-1603.
25. Raza MW, Ogilvie MM, Blackwell CC, Stewart J, Elton RA, Weir DM. Effect of respiratoty syncytial virus infection on binding of Neisseria meningitidis and Haemophilus influenzae type b to a human epithelial cell line (HEp-2). Epidemiology and Infection (accepted for publication).
26. Noah ND. Cyclic patterns and predictability in infection. Epidemiol Infect 1989; 102: 175-190.
27. Rahat A, Stewart J, Blackwell CC, Weir DM. Semi-quantitative determination of H type 1 and type 2 antigens on buccal epithelial cells and in saliva of secretor and non-secretors. Vox Sanguinis 1990; 59: 101-105.
28. Raza MW, Blackwell CC, Molyneaux P, James VS, Ogilvie MM, Inglis JM, Weir DM. Association between secretor status and respiratory viral illness. BMJ 1991; 303: 815-818.
29. Mollison PL. Red cell grouping techniques. In: Blood transfusion in clinical medicine. 7th ed. Blackwell Scientific Publications, Oxford 1983; pp 492-494.
30. Wright SD, Jong MT. Adhesion-promoting receptors on human macrophages recognize Escherichia coli by binding to lipopolysaccharide. J Exp Med 1986; 164: 1876-1888
31. Lee S. Lethal challenge of gnotobiotic weanling rats with bacterial isolates from cases of SIDS. J Clin Pathol 1987; 40: 1393-1396.
32. Newbold MJ, Malam J, McIllmurray JM, Morris JA, Telford DR, Barson AJ. Immunobiological localisation of Staphylococcal toxic shock S.T.1 antigen in SIDS. J Clin Pathol 1989; 42: 935-939.
33. Rosenstein IJ, Mizuochi T, Hounsell EF, Stoll MS, Childs RA, Feizi T. New type of adhesive specificty revealed by oligosaccharide probes in Escherichia coli from patients with urinary tract infection. Lancet 1988; ii: 1327-1330.
34. Sandford BA, Davison VE, Ramsay MA. Staphylococcus aureus adherence to influenza A-virus-infected and control cell cultures: Evidence for multiple adhesions. Proc Soc Exp Biol Med 1986; 181: 104-111.
35. McDonald KL, Osterholm MT, Hedberg CW et al. Toxic shock syndrome. A newly recognised complication of influenza and influenza-like illness. JAMA 1987; 257: 1053-1058.
36. Kremastinou J, Blackwell CC, Tzanakaki G et al. Parental smoking and carriage of Neisseria meningitidis among school children. Eighth International Pathogenic Neisseria Conference p.123. 1992.
37. Musher DM, Fainstein V. Adherence of Staphylococcus aureus to pharyngeal cells in normal subjects, smokers, staphylococcal carriers and patients with oral infections. J. Jeljaszewicz (ed): Staphylococci and Staphylococcal infections, Zbl. Bakt. suppl 10. Gustav Fisher Verlag: Stuttgart, New York 1981.
38. Cooperstock MS, Steffen E, Yolken R, Onderdonk A. Clostridium difficile in normal infants and SIDS : an association with infant formula feeding. Paediatrics 1982; 70: 91-95.
39. Gilbert R, Rudd P, Berry PJ et al. Combined effect of infection and heavy wrapping on the risk of sudden unexpected infant death. Arch Dis Child 1992; 67: 171-177.

40. Ponsonby A-L, Dwyer T, Gibbons LE, Cochrane JA, Jones ME, McCall MJ. Thermal environment and SIDS : case-control study. BMJ 1992; 304: 277-282.
41. Fleming PJ, Gilbert R, Azaz Y, Berry PJ, Rudd PJ, Stewart A, Hall E. Interaction between bedding and sleeping position in the SIDS : a population based case-control study. BMJ 1990; 301: 85-89.
42. Sunderland R, Emery JL. Febrile convulsions and cot death. Lancet 1981; ii: 176-178.
43. Guntheroth WG. Interleukin-1 as intermediary causing prolonged sleep apnoea and SIDS during respiratory infections. Medical Hypothesis 1989; 28: 121-123.

Figure Legend

Figure 1 : Binding of monoclonal anti-Lewis^{a} antibody to epithelial cells of non-secretors, secretors and Lewis^{a-b-} secretors.

Table 1. 95% confidence limits for binding of Staphylococcus aureus to non-secretor cells expressed as a percentage of binding to cells from matched secretors.

Strain	Toxin produced	Bacteria per cell 80	160	320
NCTC 8532	-	66-145	69-146	31-182
NCTC 10652	A	63-198	63-227	50-301
NCTC 10654	B	67-153	75-185	75-206
NCTC 10655	C	138-228+	107-249*	107-237*
NCTC 10656	D	61-136	79-175	67-178
NCTC 10657	A,B	110-197*	101-161*	117-165+
NCTC 11965	A,TSST-1	102-201*	92-192	81-203
40654	A	82-182	54-189	91-222
41206	B	74-147	75-172	74-158

* $P < 0.05$
\+ $P < 0.01$

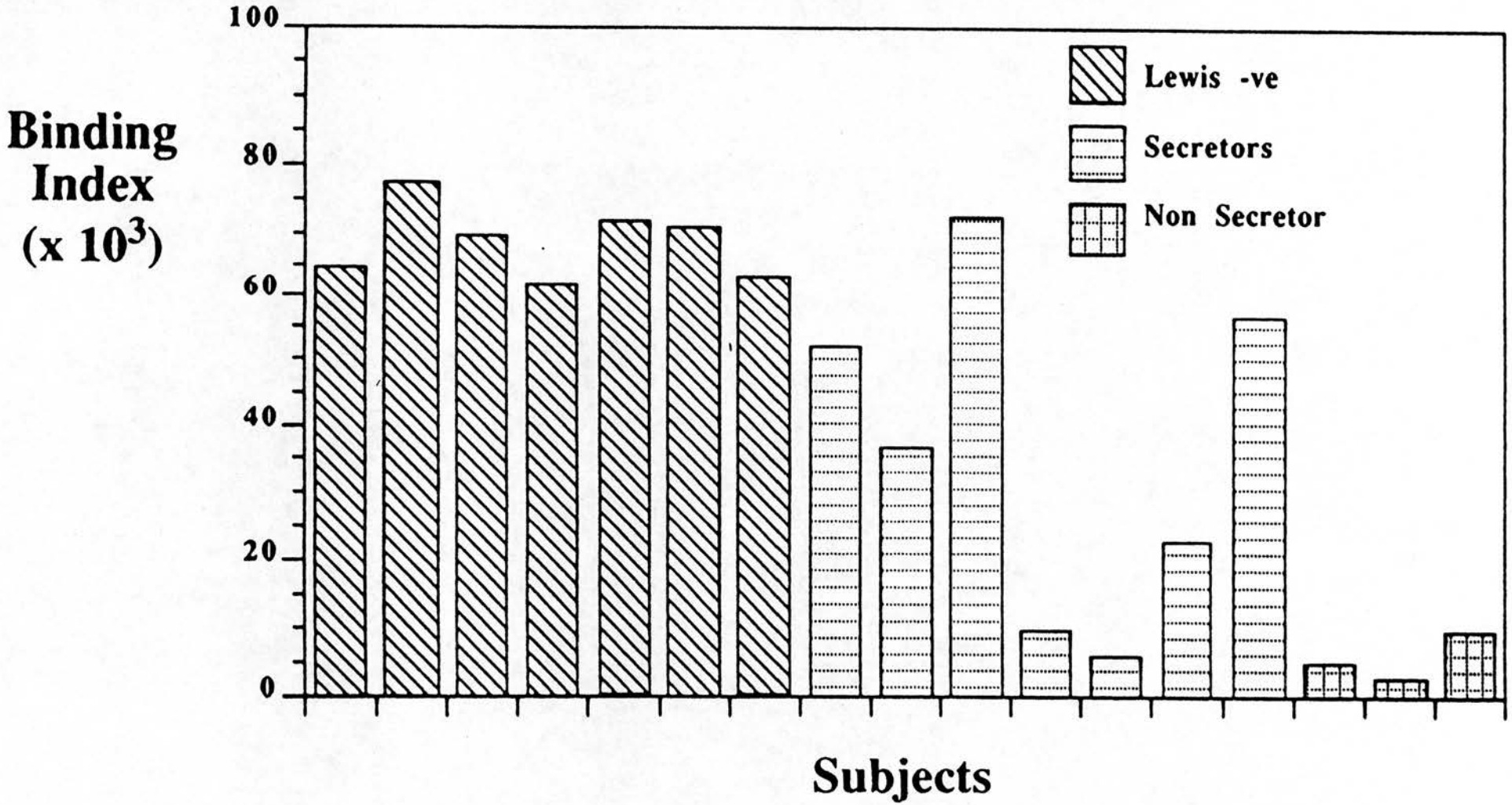


Figure 1 : Binding of monoclonal anti-Lewis^a antibody to epithelial cells of non-secretors, secretors and Lewis^{a-b-} secretors.

THE POTENTIAL ROLE OF BACTERIAL TOXINS IN SUDDEN INFANT DEATH SYNDROME (SIDS)

C.C. Blackwell, A.T. Saadi, M.W. Raza, D.M. Weir and A. Busuttil*

Department of Medical Microbiology

and

Forensic Medicine Unit, Department of Pathology

University of Edinburgh

Medical School

Teviot Place

EDINBURGH EH8 9AG

Key words : Toxins - SIDS

Running head : Bacterial toxins and SIDS

ref: ccb/mc/papers/revtony

Summary

Toxigenic bacteria have been implicated in some cases of Sudden Infant Death Syndrome (SIDS). Although there is not much evidence that Clostridia spp. are associated with SIDS in Britain, strains of Staphylococcus aureus producing pyrogenic toxins have been isolated from significant numbers of these infants at autopsy. The pyrogenic toxins, produced by some strains of group A Streptococcus pyogenes as well as staphylococci, are powerful "superantigens" that have significant physiological effects including induction of fever > 38° C. In this article, interactions between genetic and environmental factors that might enhance colonization of epithelial surfaces by toxigenic staphylococci are analyzed: infant's expression of Lewis^a antigen which acts as a receptor for some microorganisms; viral infections; the effect of mother's smoking on susceptibility to respiratory infection. Based on epidemiological findings and laboratory investigations, a hypothesis is proposed to explain how bacteria producing pyrogenic toxins might contribute to some cot deaths.

Introduction

Epidemiological studies indicate that infectious agents might be involved in some cases of Sudden Infant Death Syndrome (SIDS). At autopsy there can be evidence of minor inflammation and infection of the respiratory tract in many of these infants [1]. The deaths are more frequent during the period when maternal antibodies are declining and the immune system of the infant is immature. The risk of SIDS increases during autumn and winter months when respiratory infections are more common [2,3]. There is often a history of minor upper respiratory tract infection in these infants [4] and histological evidence of viral infection or inflammation in the lung has been reported [5,6]. Both smoking and passive exposure to cigarette smoke have been associated with increased risk of respiratory infection [7], and maternal smoking is one of the factors identified in the New Zealand studies of SIDS [8]. In some series, SIDS is more frequent in families in which socio-economic conditions are poorer, and smoking is also related to socio-economic status. In the United Kingdom, the proportion of women who smoke increases as social class decreases from "professional" to "partly skilled" or "unskilled" categories [9]. Breast feeding protects infants in this age range from gastrointestinal and respiratory illnesses, and SIDS is reported by some workers to be more frequent among bottle fed babies [8].

Microorganisms investigated for their associations with SIDS

By definition, invasive bacterial diseases are excluded from deaths diagnosed as SIDS. As a result, the role which respiratory virus infections might play in these infant deaths has been investigated [10,11], however, there is little evidence for direct associations between viral infections and SIDS [12,13]. Toxin-producing bacteria have been isolated from autopsy material. Infant botulism has been suggested to contribute to 4% of cot deaths in the United States and up to 16% of those in Sweden [14-17]. This pattern has not been observed in the United Kingdom [18]. Toxins of Clostridium difficile can produce death in monkeys with

features that are pathologically consistent with SIDS [19]. These bacteria and enterotoxigenic Escherichia coli have been isolated from a few infants [20,21], however, the evidence for involvement of intestinal toxigenic bacteria has been inconsistent.

One of the more recent hypotheses regarding sudden infant death syndrome (SIDS) is that nasopharyngeal colonization by toxigenic strains of Staphylococccus aureus might contribute to some of these cases [22]. These bacteria remain localized on the mucus membranes but their toxins disseminate into the circulation. In one series, these bacteria were isolated from the nasopharynx of approximately 40% of SIDS infants [23]. The toxic shock syndrome toxin of S. aureus can kill a previously healthy adult, so it might easily kill a small infant. Pyrogenic toxins are produced by some strains of S. aureus and also by some strains of group A beta-haemolytic Streptococcus pyogenes. These families of toxins are associated with staphylococcal food poisoning, toxic shock syndrome and also with the rash that accompanies scarlet fever. These substances are powerful "superantigens" that have significant physiological effects such as induction of fever (>38°C), possibly due to direct action on the hypothalamus or through their induction of tumor necrosis factor (TNF) and interleukin 1 by monocytes (Table 1). The bacteria can produce these toxins at temperatures between 37-40°C, but the amount of toxin produced increases with increasing temperature [24].

Factors contributing to susceptibility to infectious agents

Our research group has been investigating both genetic and environmental factors affecting susceptibility to infectious agents, in particular the secretor gene (Se) located on chromosome 19. The gene is inherited in a Mendelian dominant pattern and there are two phenotypes, secretors and non-secretors. Secretors which comprise 75-80% of most populations, have the antigens of their respective ABO blood group determinants in their body fluids. The minority 20-25% who are non-secretors do not have these antigens in their body fluids. The secretor gene is in the same linkage group as the gene for the Lewis blood group

antigens (Le), and the secretor gene controls expression of the Lewis blood group antigens. Non-secretors can produce only Lewis^a while secretors produce predominantly Lewis^b and also variable amounts of Lewis^a [25]. The distribution of the ABO and Lewis antigens on cells and in body fluids of secretors and non-secretors is summarized in Table 2.

Susceptibility to bacterial diseases and superficial yeast infections appears to be associated with the non-secretor phenotype, as does asymptomatic carriage of group A streptococci [26], meningococci [27], and Candida species [28-30]. Susceptibility to respiratory viral diseases [31] and to acquisition of the human immunodeficiency virus (HIV) through heterosexual intercourse [32] appear to be associated with the secretor phenotype .

When we examined these phenotypes among SIDS infants, the distribution of secretors and non-secretors did not differ significantly from that observed for the general population. There was, however, a high proportion of these infants in whose secretions the Lewis^a antigen was detected, 63/89 (71%) [33]. This was not unexpected; compared with adults and children over the age of 18 months, Lewis^a antigen is detected in a much higher proportion of infants. The peak incidence for the detection of Lewis^a on erythrocytes of infants is 2-3 months (80-90%) [34], coincident with a high incidence of SIDS (Figure 1).

The production of Lewis substances is due to the interactions between two fucosyl transferases, one coded for by the secretor gene and one coded for by the Lewis gene. Both these enzymes add fucose to the type 1 precursor chain from which most of the ABO and Lewis antigens in secretions is derived. If the secretor transferase adds fucose to the terminal sugar in the precursor chain, the Lewis enzyme can add fucose to the subterminal sugar to produce Lewis^b. If the Lewis enzyme adds fucose to the subterminal sugar first to produce Lewis^a, the secretor enzyme cannot use this structure as a substrate and Lewis^a is the final product [35]. In infants, the Lewis enzyme is more efficient than the secretor enzyme. As a result, infants express easily detectable amounts of Lewis^a even though the amount of this antigen might be greatly reduced as the child becomes older.

One of the hypotheses proposed to explain the higher proportion of non-secretors found among patients with bacterial or yeast infections was that there are adhesins on some strains of microorganisms that can bind to the Lewis^a antigen usually present in greater quantities on epithelial cells of non-secretors [36]. This might enhance the probability of colonization or the density of colonization by bacteria or yeasts expressing adhesins that use Lewis^a as a receptor. Evidence for this hypothesis was obtained initially from studies of Candida species [37-38], and there is evidence that the Lewis^a antigen is a receptor for the pertussis toxin [39].

If there are strains of toxigenic bacteria with adhesins that can bind to Lewis^a, infants might be easily colonized if they are exposed to these bacteria. In the age range in which SIDS occurs, they have little serum or secretory antibodies that might reduce colonization by these bacteria or activities of their toxins.

We have tested the hypothesis that there are adhesins on S. aureus that bind Lewis^a. Binding of 3 strains of S. aureus producing pyrogenic toxins (including one producing the TSST-1 toxin) to epithelial cells of non-secretors was significantly higher than to cells from secretors. As in the experiments with yeasts, pre-treatment of cells with monoclonal anti-Lewis^a significantly reduced the binding of the toxigenic strain tested. It also significantly reduced the binding of the non-toxigenic strain which bound equally well to cells of secretors or non-secretors [40].

This apparent discrepancy was solved by examining the amount of Lewis^a on epithelial cells of individual secretor and non-secretor donors. When assessed semiquantitatively by flow cytometry, binding of monoclonal anti-Lewis^a antibody to cells of non-secretors was uniformly high while binding of the antibody to cells from donors who lack the (Lewis gene) Lewis-negative was barely detectable [33]. Binding of the antibody to cells of secretors was highly variable, some as low as that observed for the Lewis-negative cells (i.e. Le^{a-b-}), some as high as that for non-secretors and some between these two extremes (Figure 2). The

binding of the bacteria was significantly correlated with the amount of anti-Lewis^a antibody detected on the epithelial cells regardless of secretor status [40].

Viral infections as predisposing factors for colonization by bacteria

Although there is no direct evidence for viruses causing cot deaths, they might be predisposing factors for colonization by toxigenic bacteria. Much of the work in this area has been done on influenza virus and superinfections by staphylococci or pneumococci [41]. We have also found that cells infected with respiratory syncytial virus (RSV) in vitro bind significantly more meningococci and type b H. influenzae [42,43]. A condition similar to toxic shock syndrome caused by staphylococci has been identified as a complication of influenza or influenza-like illness [44]. RSV is a common cause of disease among infants. Its peak prevalence occurs during the winter months [43] when SIDS is most common. By flow cytometry assays, we demonstrated that binding of a variety of bacteria including one non-toxigenic and one of the toxigenic strains of staphylococci to HEp-2 cells was significantly enhanced in RSV infected cells [40] (Figure 3).

If toxigenic staphylococci are responsible for some cot deaths, we have identified 2 factors that might contribute to increased colonization or density of colonization by these bacteria: expression of Lewis^a in this age range; infection with RSV. Since the majority of infants who become colonized by these bacteria suffer no ill effects, there must be additional factors that precipitate the chain of events leading to cot deaths. From our results and the epidemiological data reported, we have suggested the following hypothesis illustrated in Figure 4.

Exposure to and colonization by staphylococci or streptococci

Mother's smoking might enhance exposure of the infant to S. aureus. Smokers are more frequently carriers of potentially pathogenic microorganisms [30,46] and epithelial cells of smokers bind significantly more staphylococci than those of non-smokers [47]. Smoking also enhances susceptibility to respiratory viral infection, and epithelial cells from individuals with natural or experimental viral infections bound more staphylococci than those from uninfected controls [47,48]. Passive exposure to cigarette smoke decreases mucociliary clearance. The effect of breast feeding on staphylococcal or streptococcal carriage is not known, however, breast fed infants are less susceptible to toxigenic strains of Cl. botulinum [49].

Enhancement of toxin production

If an infant becomes heavily colonized with bacteria producing pyrogenic toxins and environmental factors raise the body temperature, this might increase the quantity of toxin produced. Body temperature might be increased by concurrent minor respiratory viral infection, over-wrapping or placing the infant in the prone sleeping position [50,51]. The toxin produced diffuses into the blood stream to increase the temperature of the infant and further enhance toxin production. This synergistic effect might account for the unusually high temperatures recorded for some cases of SIDS [52]. The actual cause of death might be heat shock or increased frequency or duration and depth of sleep due to induction of IL-1 which has been proposed as the link between respiratory infections and fatal sleep apnoea [51].

Acknowledgements

This work was supported by the Scottish Cot Death Trust.

Legends for figures

Figure 1

Detection of Lewis^a antigen on cells of infants and incidence of SIDS.

Figure 2

Variability in binding of monoclonal anti-Lewis^a to epithelial cells of non-secretors, secretors and Lewis-negative individuals.

Figure 3

Binding of Staph. aureus to HEp-2 cells and HEp-2 cells infected with RSV.

Figure 4

Factors proposed to affect colonization of infants by toxigenic Staph. aureus and to precipitate events leading to SIDS.

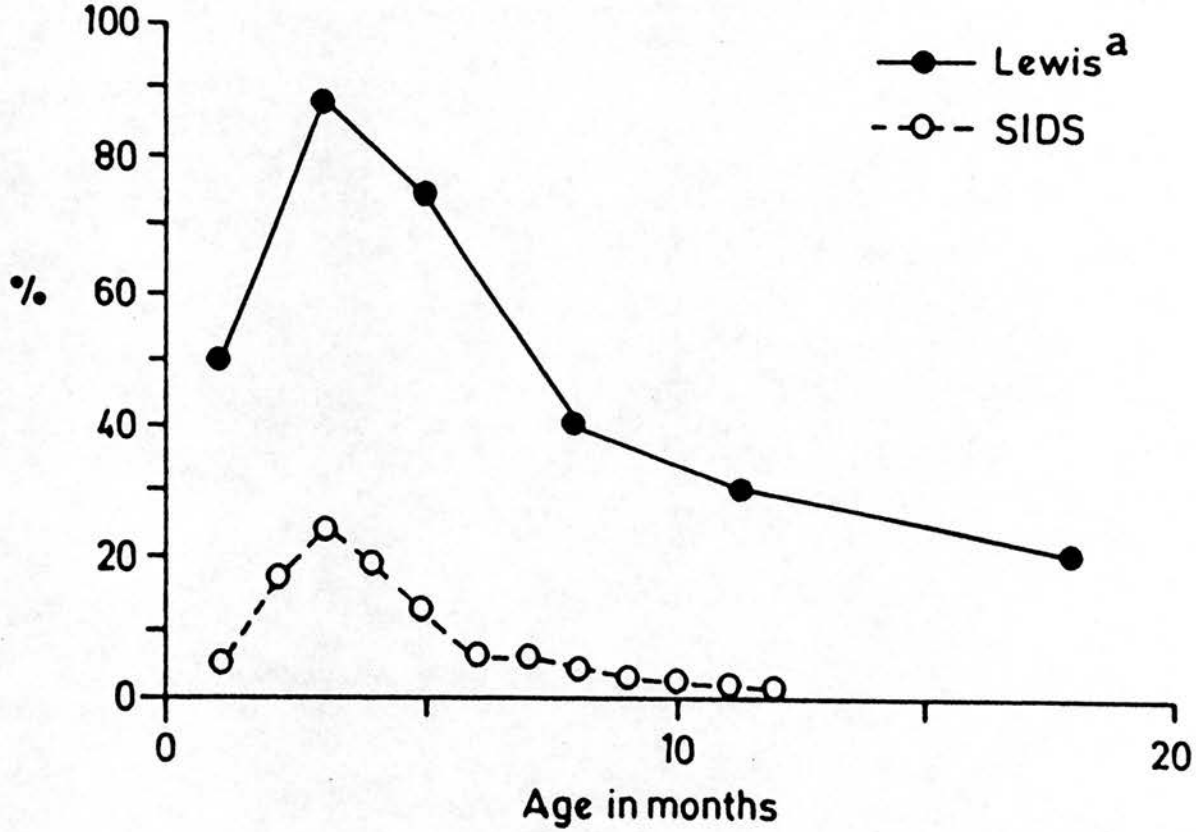
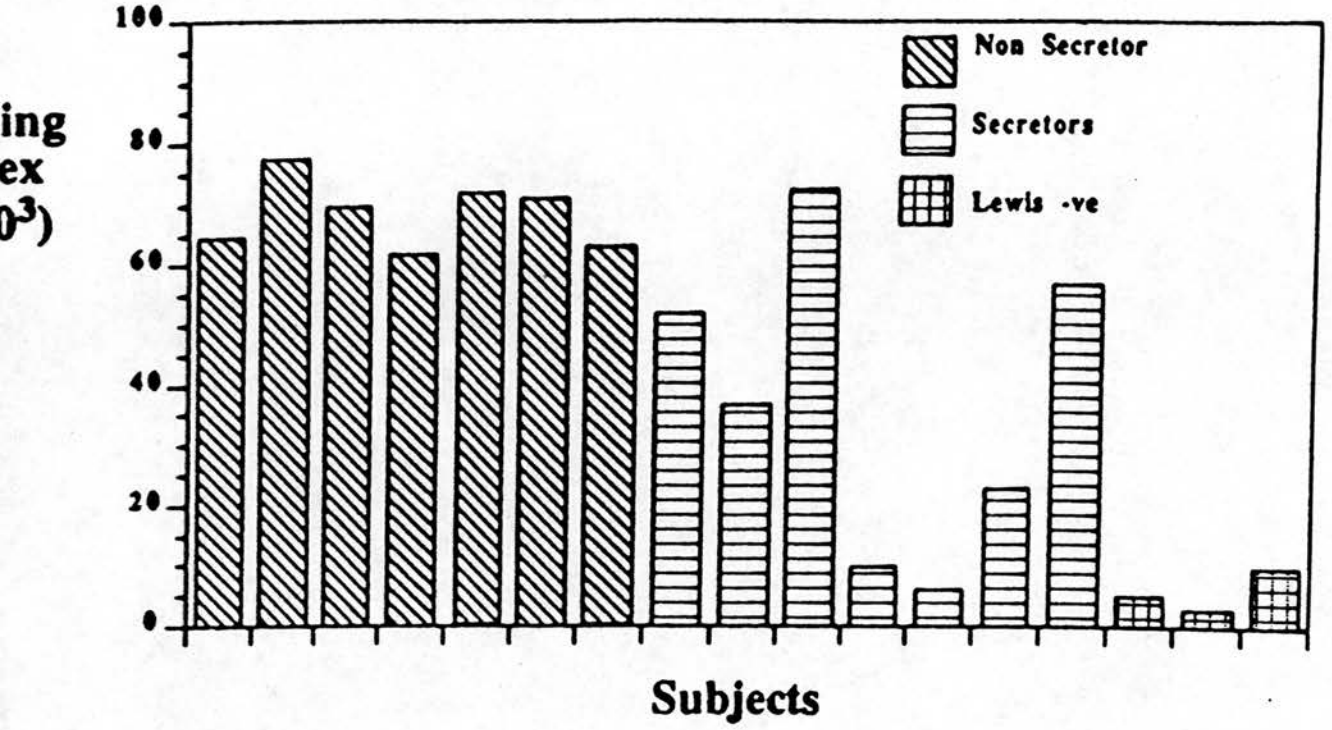


FIG 1

Fig. 2


ing
ex
0^3)
100
80
60
40
20
0
Non Secretor
Secretors
Lewis -ve
Subjects

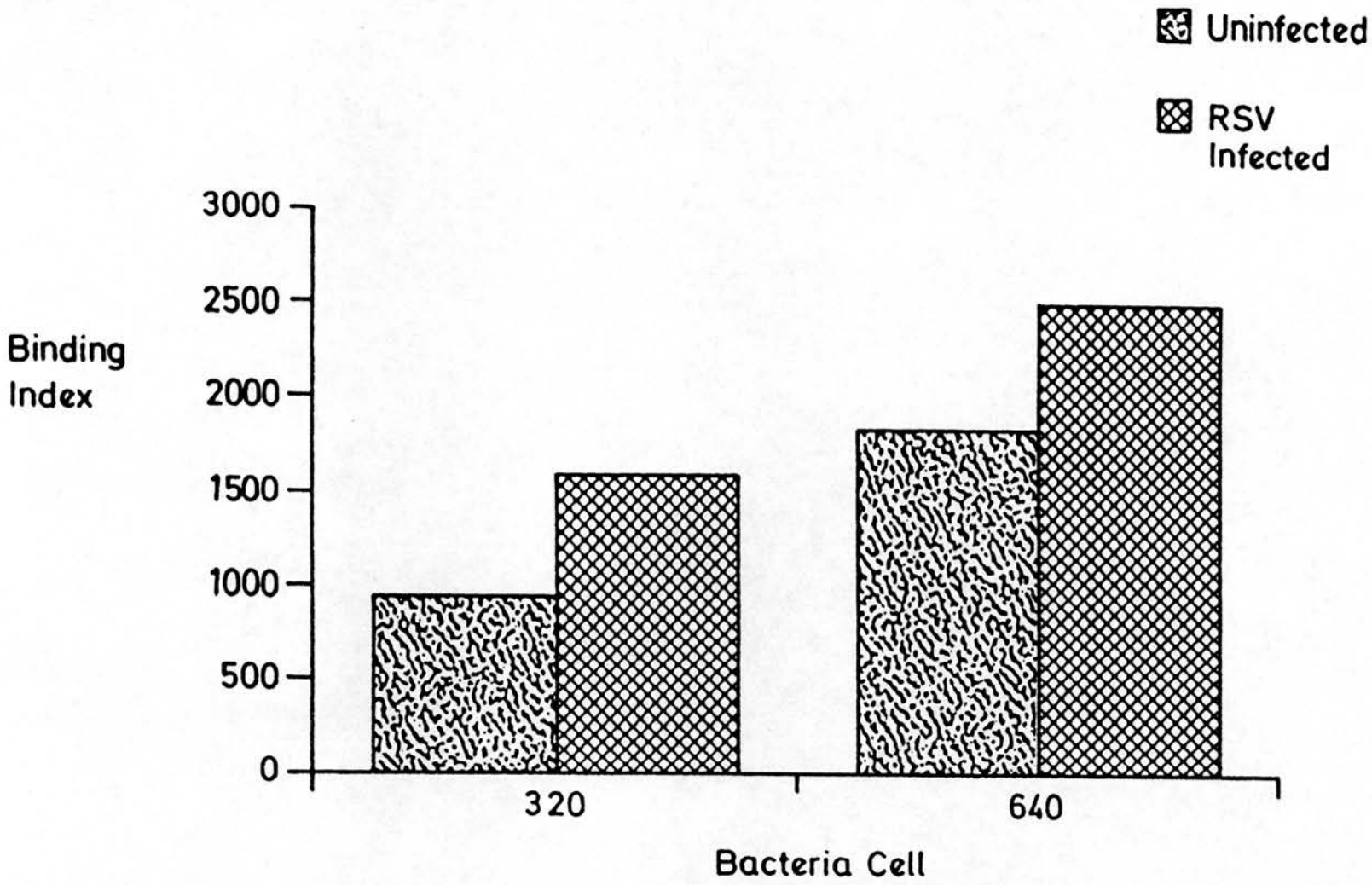
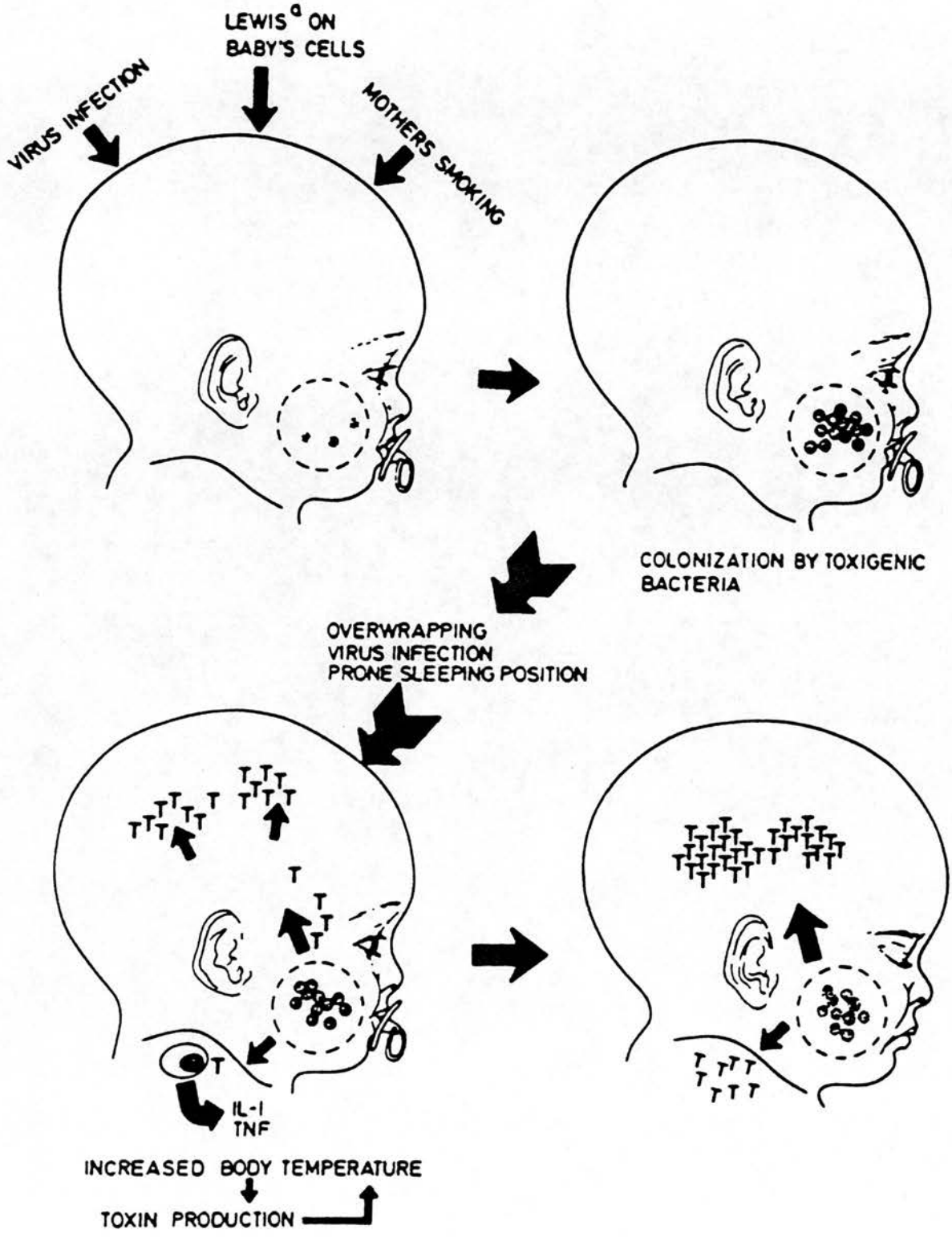

Uninfected
RSV Infected
Binding Index
3000
2500
2000
1500
1000
500
0
320
640
Bacteria Cell

Fig 3

Fig. 4


LEWIS a ON
BABY'S CELLS
VIRUS INFECTION
MOTHERS SMOKING
COLONIZATION BY TOXIGENIC
BACTERIA
OVERWRAPPING
VIRUS INFECTION
PRONE SLEEPING POSITION
IL-1
TNF
INCREASED BODY TEMPERATURE
TOXIN PRODUCTION

REFERENCES

1. Berry PJ, Keeling JW (1989). The investigation of sudden infant death in infants. In: Anthony PP, MacSween RND (eds) Recent advances in histopathology. Churchill Livingstone, London, pp 251-279.
2. Froggat P, Lynas MA, Mackenzie G (1971). Epidemiology of sudden unexpected death in infants in N. Ireland. Br J Paediatr Soc Med 25: 119-134.
3. Carpenter RG, Gardner A (1982). Variations in unexpected infant death rates relating to age, sex and season. Studies on medical and population subjects. HM Stationery Office, London. 45, 23-31.
4. Valdes-Dapena MA (1967). Sudden and unexpected death in infancy: a review of the world literature 1954-1966. Pediatrics 39: 123-138.
5. Entrup M, Brinkmann B (1990). Histologische Lungenbefunde beim plötzlichen Kindstod. Z. Rechtsmed 103: 425-433.
6. Berg VS, Kijewski S (1984). In: Wilske J (ed) Der plötzliche Säuglingstod (SIDS). Histologische Befunde an 244 Fällen von plötzlichem Säuglingstod im norddeutschen Raum. Springer-Verlag, Berlin, Heidelberg, New York, Tokyo pp 153-160
7. Bonham GS, Wilson RW (1989). Childrens health in families with cigarette smokers. Am J Public Health 71: 290-293.
8. Mitchell EA (1991). Cot death: should the prone sleeping position be discouraged? J Paediatr Child Health 27: 319-321.
9. Wald N, Kiryluk S, Darby S, Doll R, Pike M, Peto R (1988). UK smoking statistics. Oxford University Press, Oxford.
10. Scott DJ, Gardner PS, McQuillan J, Stanton AN, Downham MAPS (1978). Respiratory viruses and cot death. BMJ ii: 12-13.
11. Williams AL, Uren EC, Bretherton L (1984). RSV and SIDS. BMJ 288: 1491-1493.
12. Fleming K (1992). Upper airways inflammation and detection of viral nucleic acid in SIDS. J Clin Pathol (in press).

13. Howatson AG (1992). Alpha interferon and enteroviral infection in SIDS. J Clin Pathol (in press).

14. Arnon SS, Midura TF, Damus K, Wood RM, Chin J (1978). Intestinal infection and toxin production by Clostridium botulinum as one cause of sudden infant death syndrome. Lancet: 1273-1276

15. Arnon SS, Damus K, Chin J (1981). Infant botulism: epidemiology and relation to sudden infant death syndrome. Epidemiol Rev 3: 45-66.

16. Sonnabend OAR, Sonnabend WFF, Krech U, Molz G, Sigrist T (1985). Continuous microbiological and pathological study of 70 sudden and unexpected infant deaths: toxigenic intestinal Clostridium botulinum infection in 9 cases of sudden infant death syndrome. Lancet: 237-241.

17. Anonymous Editorial (1986). Infant botulism. Lancet: 1256-1257.

18. Turner HD, Brett EM, Gilbert RJ, Ghosh AC, Liebeschuetz HJ (1978). Infant botulism in England. Lancet: 1277-1278.

19. Arnon SS, Mills DC, Day PA, Henrickson RV, Sullivan NM, Wilkins JD (1984). Rapid death by infant rhesus monkeys injected with Clostridium difficile toxins A & B: physiologic and pathologic basis. J Pediatr 104: 34-40.

20. Gurwith MJ, Langston C, Citron DM (1981). Toxin producing bacteria in infants. Lack of an association with sudden infant death syndrome. Am J Dis Child 135: 1105-1106.

21. Cooperstock MS, Steffen E, Yolken R, Onderdonk A (1982). Clostridium difficile in normal infants and sudden infant death syndrome: an association with infant formula feeding. Pediatrics 70: 91-95.

22. Morris JA, Haran D, Smith A (1987). Hypothesis: common bacterial toxins are a possible cause of the sudden infant death syndrome. Med Hypotheses 22: 211-222.

23. Telford DR, Morris JA, Hughes P, Conway AR, Lee S, Barson AJ, Drucker DB (1989). The nasopharyngeal bacterial flora in the sudden infant death syndrome. J Infect 18: 125-130.

24. Bohach GA, Fast DJ, Nelson RD, Schlievert PM (1990). Staphylococcal and streptococcal pyrogenic toxins involved in toxic shock syndrome and related illnesses. Crit Rev Microbiol 17: 251-272.

25. Race RR, Sanger R (1968). Blood Groups in Man, 5th edn. Blackwell Scientific Publications, Oxford, pp 291-330.

26. Haverkorn MK, Goslings WRO (1969). Streptococci, ABO blood groups and secretor status. Am J Hum Genet 21: 360-375.

27. Blackwell CC, Weir DM, James VS, Todd WTA, Banatvala N, Chaudhuri AKR, Gray HG, Thomson EJ, Fallon RJ (1990). Secretor status, smoking and carriage of Neisseria meningitidis. Epidemiol Infect 104: 203-209.

28. Burford-Mason AP, Welsby JCP, Willoughby JMT (1988). Oral carriage of Candida albicans, ABO blood group and secretor status inhealthy individuals. J Med Vet Mycol 26: 49-56.

29. Aly FZ, Blackwell CC, Mackenzie DAC, Weir DM, Elton RA, Sofaer JA, Clarke BF (1991). Chronic atrophic oral candidiasis among patients with diabetes mellitus - the role of secretor status. Epidemiol Infect 106: 355-363.

30. Aly FZM, Blackwell CC, Mackenzie DAC, Weir DM, Clarke BF (1992). Factors influencing oral carriage of yeasts among individuals with diabetes mellitus. Epidemiol Infect (in press).

31. Raza MW, Blackwell CC, Molyneaux P, James VS, Ogilvie MM, Inglis JM, Weir DM (1991). Association between secretor status and respiratory viral illness. BMJ 303: 815-818.

32. Blackwell CC, James VS, Davidson S, Wyld R, Brettle RP, Robertson RJ, Weir DM (1991). Secretor status and heterosexual transmission of HIV. BMJ 303: 825-826.

33. Blackwell CC, Saadi AT, Raza MW, Stewart J, Weir DM (1992). Susceptibility to infection in relation to sudden infant death syndrome. J Clin Pathol (in press).

34. Issit PD (19..) Applied blood group serology, 3rd edn Montgomery, Miami pp 169-191.

35. Watkins WM, Greenwell P, Yates AD, Johnson PH (1988). Regulation of expression of carbohydrate blood group antigens. Biochimie 70: 1597-1611.

36. Blackwell CC (1989). The role of ABO blood groups and secretor status in host defences. FEMS Microbiol Immunol 47: 341-350.

37. May SJ, Blackwell CC, Weir DM (1989). Lewis^a blood group antigen of non-secretors: a receptor for Candida blastospores. FEMS Microbiol Immunol 45: 407-410.

38. Tosh FD, Douglas LJ (1991). Effect of blood group and secretor status on the adhesion of Candida albicans to mucosal surfaces. In: Umbay ET et al (eds). Candida and Candida mycosis. Plenum Press, New York, pp. 127-130.

39. Van't Wout J, Burnette WN, Mar VL, Razdzinski E, Wright SD, Tuomanen E (1992). Role of carbohydrate recognition domains of pertussis toxin in adherence of Bordetella pertussis to human macrophages. Infect Immun 60: 3303-3308.

40. Saadi AT, Blackwell CC, Raza MW, James VS, Stewart J, Elton RA, Weir DM (1992). Factors enhancing adherence of toxigenic staphylococci to epithelial cells and their possible role in sudden infant death syndrome. Epidemiol Infect (Submitted for publication).

41. Plotkowski M-C, Puchelle E, Beck G, Jaquet J, Hannoun C (1984). Adhernece of type 1 Streptococcus pneumoniae to tracheal epithelium of mice infected with influenza A/PRB virus. Am Rev Respir Dis 134: 1040-1044.

42. Raza MW, Ogivlie MM, Blackwell CC, Stewart J, Elton RA, Weir DM (1991). Infection with respiratory syncytial virus enhances binding of Neisseria meningitidis to HEp-2 cells. 120th meeting Society for General Microbiology, Nottingham, UK. Abstract Booklet p.29.

43. Raza MW, Ogilvie MM, Blackwell CC, Stewart J, Elton RA, Weir DM. Effect of respiratory syncytial virus infection on binding of Neisseria meningitidis and type b Haemophilus influenzae to human epithelial cell line (HEp-2). Epidemiol Infect (accepted for publication).

44. McDonald KL. Osterholm MT, Hedberg CW, Schrock CG, Peterson GF, Jentzen HM, Leonard SA, Schlievert PM (1987). Toxic shock syndrome. A newly recognised complication of influenza and influenza-like illness. JAMA 257: 1053-1058.

45. Noah ND (1989). Cyclic patterns and predicatibility in infection. Epidemiol Infect 102: 175-190.

46. Blackwell CC, Tzanakaki G, Kremstinou J, Weir DM, Vakalis N, Elton RA, Mentis A (1992). Factors affecting carriage of Neisseria meningitidis among Greek military recruits. Epidemiol Infect 108: 441-448.

47. Musher DM, Fainstein V (1981). Adherence of Staphylococcus aureus to pharyngeal cells from normal subjects, smokers, staphylococcal carriers and patients with viral infection. In: J. Jeljaszewicz Staphylococci and staphylococcal infections. Zentrabl. Bakt. Suppl 10: 1011-1016.

48. Ramírez-Ronda CH, Fuxench-Lopez A, Nevarez M (1981). Increased bacterial colonisation during viral illness. Arch Intern Med 141: 1599-1603.

49. Arnon SS (1989). Breast feeding and toxigenic intestinal infections: missing links in crib death? Rev Infect Dis Suppl 6: S193-S201.

50. Ponsoby A-L, Dwyer T, Gibbons LE, Cochrane JA, Jones ME, McCall MJ (1990). Thermal environment and SIDS : case-control study. BMJ 304: 277-282.

51. Fleming PJ, Gilbert R, Azaz Y, Berry PJ, Rudd PJ, Stewart A, Hall E (1990). Interaction between bedding and sleeping position in the SIDS : a population based case-control study. BMJ 301: 85-89.

52. Sunderland R, Emery JL (1981). Febrile convulsions and cot death. Lancet: 176-178.

53. Guntheroth WG (1989). Interleukin-1 as intermediary causing prolonged sleep apnoea and SIDS during respiratory infections. Med Hypotheses 28: 121-123.

Parental Smoking and Carriage of *Neisseria meningitidis* Among School Children

J. Kremastinou

Athens School of Public Health, Athens Greece

C.C. Blackwell, D.M. Weir, R.A. Elton, G. Chronas

Departments of Medical Microbiology and Public Health Sciences, University of Edinburgh, Edinburgh, Scotland

G. Tzanakaki, A. Mentis

National Meningitis Reference Laboratory, Hellenic Institute Pasteur, Athens, Greece

C. Kallergi

Aglaia Kyriakou Paediatric Hospital, Athens, Greece

Since 1989 there has been a steady increase in the numbers of cases of meningococal disease reported to the Ministry of Health in Athens (1). In December 1990 and January 1991 primary (324) and secondary (713) pupils in two areas of Athens were screened to determine if there was an increase in the rate of carriage of Neisseria meningitidis and to assess genetic and environmental factors previously associated with carriage of meningococci in northern European populations: age [2]; sex (2,3); secretor status (4); smoking or passive exposure to cigarette smoke (1,4,5,6); lower socioeconomic conditions (2); upper respiratory tract infections (7,8).

The effect of cigarette smoke was of particular interest because of the strong association between smoking and carriage observed among Greek military recruits (1) and the proportion of recruits who were smokers

(62%) was higher than than among men of similar age in Britain (40%) (9).

Results

Children in two areas of Athens were screened for carriage of meningococci. In area A socioeconomic indicators, housing costs and the educational level of the parents (Table I) were significantly lower. Over half the parents in area A had less than 7 years of formal education (category 1); only 5% had received technical training (category 4) and 2% had attended university (category 5).

Table I. Educational (socioeconomic) Levels of Parents in Areas A and B

	Category %				
	1	2	3	4	5
Area					
A (n=1122)	52	21	19	5	2
B (n=937)	18	14	31	8	29

($P < 0.0005$)

The overall carriage rate was 60/1038 (5.8%), for area A 30/565 (5.3%) for Area B 30/473 (6.3%).

Univariate analysis found no association between carriage and sex, viral infection or secretor status. There was no associaton between carriage and educational level of either parent or numbers of individuals per household in either area.

Carriage was increased among secondary pupils who smoked, but this was significant only in Area A: 5/30 smokers (17%) were carriers compared with 16/352 non-smokers (4.5%) ($P < 0.025$).

Carriage rates were higher among children from households where adults smoked. Only 6/214 (2.8%) of children from non-smoking households were carriers compared with 28/360 (7.8%) ($P < 0.025$) from households where the mother smoked or if grand parents smoked (11/129, 8.5%, $P < 0.05$). The increase associated with the father's smoking was not significant (38/571, 6.7%). When figures were assessed after elimination of children who smoked, 20/38 (52.6%) carriers who were non-smokers had mothers who smoked. In area A the proportion of father's who smoked was significantly higher (345/560, 61%) than in Area B (223/472, 47%) ($P < 0.0005$). These differences were not observed for mothers in Area A (33%) compared with Area B (38%).

Multiple logistic regression analysis identified only age ($P < 0.01$) and maternal smoking ($P < 0.05$) to be significant factors associated with carriage. Older children and those whose mothers smoked were more likely to be carriers (Table II).

Table 2. Carriage Rates in Children Classifed by Age and Maternal Smoking

	Age		
	no. (%)		
	5-9	10-14	15-19
mother smoker	1/66 (1.5)	5/99 (5.1)	22/195 (11.3)
mother non-smoker	5/118 (4.2)	8/321 (3.5)	19/329 (5.8)

Discussion

The proportion of carriers among school children was within the expected range, 5-10%. Several factors associated with carriage in studies in northern Europe were not identified in this study. The two significant factors were age and mother's smoking.

The isolation rate in different age bands followed a pattern similar to that found in the Stonehouse study (2); there were more carriers the 15-19 year age range.

In contrast to the Stonehouse survey in which isolation rates of both the outbreak strain and non-outbreak strains were greater in the parts of the town where socioeconomic indicators were lower, there was no difference between the isolation rates for the two areas of Athens. Patterns of smoking in the two countries might explain these differences. As in the United Kingdom (9), smoking among Greek men is correlated socioeconomic level. A similar pattern is observed for British women, but not for Greek women; among the mothers in the study, there was no significant association between smoking and educational level (Table III).

Table III. Sex, Smoking and Educational Level of Parents

	educational category %				
	1	2	3	4	5
women					
smoker (n=329)	35	38	39	30	25
men					
smoker (n=568)	62	59	55	53	38

women ($P > 0.05$)
men ($P < 0.0005$)

The observation that carriage was also associated with smoking by adults other than the father might reflect the structure of many Greek families; grandparents often live with families with young children and are closely involved in child care.

References

1. Blackwell, C.C., Tzanakaki, G., Kremastinou, J. et al. 1992. Epidemiol. Infect. 108: 441.

2. Cartwright, K.A.V., Stuart, J.M., Jones, D.M. et al. 1987. Epidemiol. Infect. 99: 591.

3. Olcen, S.F., Djurhuus, B., Rasmussen, K. et al. 1991. Epidemiol. Infect. 106: 445.

4. Blackwell, C.C., Weir, D.M., James, V.S. et al. 1990. Epidemiol. Infect. 104: 203.

5. Stuart, J.M., Cartwright, K.A.V., Dawson, J.A. et al. 1988. Commun. Med. 10: 139.

6. Stuart, J.M., Cartwright, K.A.V., Robinson, P.M. et al. 1989. Lancet ii: 723.

7. Krakinski, K., Nilson, J.D., Butler, S. et al. 1987. Am. J. Epidemiol. 125: 499.

8. Moore, P.S., Hierholzer, J., Dewitt et al. J. Am. Med. Assoc. 246: 1271.

9. Wald, N., Kiryluk, S., Darby, S. et al. 1988 UK Smoking Statistics Oxford University Press.

Carriage of *Neisseria meningitidis* Among Military Recruits and School-aged Children in Greece

J. Kremastinou
Athens School of Public Health, Athens Greece

G.Tzanakaki, A. Mentis
National Meningitis Reference Laboratory, Hellenic Institute Pasteur, Athens Greece

C.C. Blackwell, D.M. Weir, R.A. Elton
Departments of Medical Microbioloogy and Public Health Sciences, University of Edinburgh, Edinburgh Scotland

Introduction

Following the epidemic of meningitis in the 1960's, there was a steady decrease in the numbers of cases reported to the Ministry of Health in Athens until 1988. In 1989, the numbers began to increase, and members of the Athens School of Public Health and the Hellenic Institute Pasteur invited our group to help organize studies on meningococcal carriage and the characteristics of *Neisseria meningitidis* circulating in the Greek population (1) . The last major surveys on meningococci had been undertaken before serotype and subtype antibodies were available (1); and, there was no information on antibiotic sensitivities. In contrast to northern Europe, there had been no studies on genetic and environmental actors affecting carriage of meningococci (3,4,5)

Environmental factors suggested to affect meningococcal carriage include smoking or passive exposure to cigarette smoke (4,6). Several groups have suggested viral infections might be predisposing factors for

meningococcal disease (7,8); and viral infections are predisposing factors or carriage of potentially pathogenic bacteria (9). The objectives of the surveys were to answer the following questions:

1. Is there an increase in the proportion of carriers among recruits or school children?
2. Are factors associated with carriage in Greece the same as those in northern Europe?
3. Can the available serotype and subtype reagents differentiate Greek strains?
4. Are the antigenic phenotypes associated with outbreaks in north and west European countries prevalent among patients or carriers in Greece?
5. Are there higher proportions of penicillin-insensitive strains or strains resistant to other antibiotics compared with northern countries?

Results

Carriage of meningococci among military recruits

Greek military recruits (993) from two camps, one near Athens (Haidari) (Camp A) and one near Avlona 50 km north of Athens (Camp B), were examined for carriage of meningococci during the third week of July 1990. The specimens and information obtained from the recruits are summarized in Table I.

Table I. Specimens and Information Obtained from Recruits

Subjects:	993 recruits in 2 military camps
Specimens:	throat swabs plated directly to MNYC
	10 ml blood
	5-10 ml saliva
Questionnaire:	age
	educational level
	smoking habits
	place of residence
	recent illness

Meningococci were isolated from 247 (25%) of the recruits; however, between the two camps, there were significant differences in the carriage rates and other factors examined. The carriage rate for Camp B (30%) was significantly higher than that for camp A (18%) ($P < 0.0005$); and in Camp B there were significantly higher proportions of non-secretors ($P < 0.0005$), those with less than 7 years of schooling ($P < 0.0005$), younger recruits (<19 years of age) ($P < 0.001$) and heavy smokers (> 30 cigarettes / day) ($P < 0.0005$) (1,9).

By univariate analysis, smoking and the number of cigarettes smoked per day were associated with carriage of meningococci ($P < 0.0005$). By multivariate analysis, carriage was associated with smoking ($P < 0.001$), the younger age group (<19 years) ($P < 0.01$) and the camp ($P < 0.01$). (1,9).

In Camp B, although the carriage rate was 30%, it was 40% among recruits that had viral infections. These studies were carried out in July when the respiratory infections are not common. They were repeated in the same camps in January 1991 to examine carriage rates at a time when respiratory infections were more common. Although the proportion of

recruits with symptoms of colds was increased, the overall proportion of carriers was increased by only 2% from 25 to 27% (Table II).

Table II. Upper Respiratory Tract Infection (URTI) and Carriage of Meningococci

	July	January
Camp A		
no.	432	491
	%	
URTI	4.9	46.4
carriers	18	24
Camp B		
no.	561	500
	%	
URTI	6.7	34
carriers	30	31

Carriage of meningococci among school-age children

In December 1990 and January 1991 school children in two areas of Athens were screened in a survey similar to that carried out among the recruits (Table III)

Table III. Specimens and Information Obtained from School-age Children

Subjects: 1038 primary and secondary pupils in two areas of Athens; In Area A socioeconomic indicators were lower than in B

Specimens: Throat swabs plated directly to MNYC agar
5-10 ml saliva

Questionnaire: age
school
class
recent illness
educational level of mother and father
no. members of household
who smokes in household

In Area A, the housing costs were lower and the educational level of both parents were significantly lower than in Area B ($P < 0.0005$). The carriage rates for the two areas were similar, A = 5.3% and B = 6.3%.

Univariate analysis found no association between age, sex, secretor status, area of residence, educational level of parents, numbers of members per family, school attended or respiratory tract infection. Carriage was associated with the mother's smoking or smoking by a member of the household other than the father. By multivariate analysis, the age of the child and mother's smoking were found to be significantly associated with carriage (Table IV).

Table IV. Factors Associated with Carriage among School-age Children

	Factor	P
univariate analysis	smoking	
	mother	<0.025
	father	ns
	other	<0.05
multivariate analysis	age (15-19)	<0.01
	mother's smoking	<0.05

Among fathers, smoking was significantly more prevalent among those with less formal education ($P < 0.0005$), a pattern similar to that observed for men in the United Kingdom (10). Although a similar association between smoking an socioeconomic indicators is found among women in Britain, this was not observed among women in Greece; there was no association between educational level and smoking (11). If the mother's smoking is a significant factor associated with carriage, this might explain the similar isolation rates for the two areas of Athens. The observation that carriage was associated with smoking by adults other than the father might reflect the structure of many Greek families; grandmothers often live with families with young children and and are closely involved in child care.

Antigenic characteristics of meningococcal isolates from patients and carriers

The majority of isolates from patients or carriers did not react with any of the monoclonal serotype reagents examined; however, most reacted with one or more of the subtype reagents (12,13). The results suggest that new serotype reagents need to be developed for epidemiological studies in Greece and possibly other countries in eastern Europe. Nearly 600 isolates have been examined; only 1 strain obtained from a school child in Athens expressed antigenic combinations associated with outbreaks in northern Europe. This was a B:4:P1.15 strain, but it was

not resistant to sulphonamide. The predominant serotype/subtype combination found among patient isolates was 2b:P1.10; however, only 4% of isolates from children who were carriers expressed this phenotype but none of those from the recruits (12).

Antibotic resistance

In 1991, the proportion of penicillin insensitive (PI) isolates among recruits who were carriers (37%) was lower than that among patient isolates (48%) but higher than that obtained from school children who were carriers (19%). The proportion of isolates resistant to sulphamethoxazole, tetracycline erythromycin and cefaclor wre higher in the recruits compared with the children who were carriers (14,15). February 1992, the proportion of PI isolates had increased to 37% as had all the other resistances except tetracycline.

Discussion

The results are discussed in the context of the questions posed in the Introduction. The proportion of carriers was not above that expected for the two populations examined, military recruits and school children. In both populations, smoking or exposure to cigarette smoke were factors associated with carriage as was age. In both populations, the rate of carriage was highest in the 15-19 year age range; similar results were reported for the Stonehouse survey (16). While the subtype reagents were able to differentiate the strains from both carriers and patients, the serotype antibodies did not react with the majority of strains. Only 1 out of nearly 600 isolates examined expressed antigenic phenotypes associated with epidemics of group B meningococcal disease in northern Europe. This indicates new serotype reagents need to be developed for studies in Greece and that vaccines presently under trail in Norway would not be appropriate for this population. Penicillin insensitive isolates, particularly those from carriers, are more common in southern European countries such as Greece and Spain (17) where

antibioitic usage is less well regulated.

References

1. Blackwell, C.C., Tzanakaki, G. Kremastinou J. 1992. Epidemiol. Infect. 108: 441.

2. Kalapothaki, V. Vassilliadis, P., Kaklamani, E. et al. 1979. Annal. Microbiol. (Inst. Pasteur). 120B: 43.

4. BlackwellC.C., Weir, D.M., James, V.S. et al., 1990. Epidemiol. Infect. 104: 203.

5. Olcen, S.F., Djurhuus, B., Rasmussen, K. 1991. Epidemiol. Infect. 106: 445.

6. Stuart, J.M., Cartwright, K.A.V., Dawson, J.A. 1988. Community Med. 10:139.

7. Moore, P.S., Hierholzer, J., Dewitt et al. 1990. J. Am. Med. Assoc. 246: 1271.

8. Cartwright et al., 1991

9. Blackwell, C.C., Weir, D.M., Elton, R.J. et al. (this volume).

10. Wald, N., Kiryluk, S., Darby, S. et al. 1988. UK Smoking Statistics. Oxford University Press.

11. Kremastinou, J., Blackwell, C.C., Weir, D.M. et al. (this volume)

12. Tzanakaki, G., Blackwell, C.C., Kremastinou, J. et al. (in press)

13. Tzanakaki, G., Mentis, A., Kremastinou, J. et al. (this volume).

14. Tzanakaki, G., Blackwell, C.C., Kremastinou, J. et al. 1992. Epidemiol. Infect. 108: 449.

15. Tzanakaki, G., Blackwell, C.C., Weir, D.M. et al. (this volume).

16. Cartwright, K.A.V., Stuart, J.M., Jones, D.M. et al., 1987. Epidemiol. Infect. 99: 591.

17. Saez-Nieto, J.A., Vazquez, J.A., Macros, C. 1990. Lancet ii: 54.